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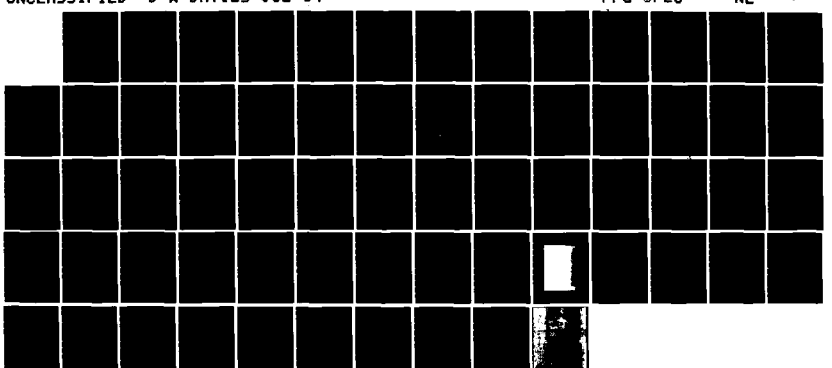

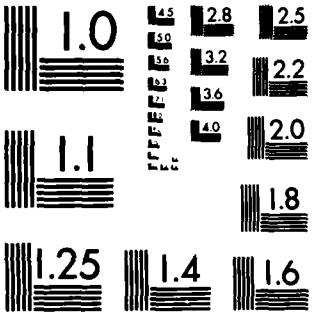

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INHALATION TOXICOLOGY OF FOG OIL OBSCURANT

PHASE I: INHALATION EXPOSURE FACILITY

David W. Davies

JULY 1984

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701

Army Project Order 1810

Toxicology Branch
Inhalation Toxicology Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Project Officer: Mary C. Henry, Ph.D.
Health Effects Research Division
U.S. ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701

AD-A144 875

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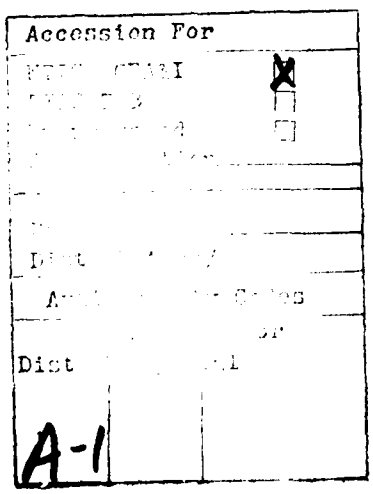

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Exposure chamber	Inhalation	RAM-1
Exposure facility	Lubricating oil	Remote control
Fog oil		Smoke

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

An inhalation exposure facility was developed, tested, and operated to expose rodents to a fog oil obscurant. The criteria considered in establishing the facility were: adequate chamber and exposure facility ventilation, control of chamber temperature, aerosol generation and control, aerosol monitoring, aerosol homogeneity, and aerosol removal. Conditioned air was supplied to the laboratory area through volume dampers controlled by -

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thermostats providing individual air conditioning for each room. Room air was filtered before entering the chambers. Chamber temperature was monitored using a thermocouple and digital display thermometer and a control circuit provided chilled water to the chamber inlet air coil if the temperature was higher than desired. A manually adjusted blower pushed air through the inlet air coil, particulate and gas filters, and into a two-path generator/bypass loop. The chamber atmosphere was exhausted through a coalescing filter. The oil was injected onto a Vycor glass heating element in an inert nitrogen atmosphere. The heater was controlled by a dual set point temperature controller ensuring duplicate temperature protection for the generator heater. The oil was vaporized in an inert nitrogen atmosphere to prevent combustion. As the oil vapor/nitrogen mixture flowed through the generator, the temperature was maintained by a heat tape to prevent premature condensation. The mixture was injected into the generator portion of the dual path chamber inlet air duct. The oil vapor quickly condensed to form the smoke when diluted with chamber supply air. The aerosol passed through an orifice plate used to monitor flow and to enhance stream mixing. The aerosol entered at the top of the chamber and was deflected to the chamber perimeter by a conical baffle. Two perforated plates at the top of the chamber promoted mixing and uniform aerosol distribution. Average droplet size of the aerosol, measured by a 7-stage cascade impactor, was approximately 1 to 1.3 μm MMAD with a geometric standard deviation of 1.5. Chamber aerosol concentrations were monitored using Real-time Aerosol Monitors (RAM-1). These sensors were calibrated to gravimetric fflter samples. Chamber aerosol concentrations between 0.4 and 10.0 mg oil/L air were generated. Control and monitoring of all chamber operating parameters were conducted from a remote control room. This isolation provided a high level of safety for operating personnel.

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EXECUTIVE SUMMARY

The U.S. Army is interested in using visual obscurant smokes for peacetime personnel training missions. This interest, combined with a desire to protect troop health, led to the construction of a small-animal inhalation exposure facility to conduct exposures of laboratory animals to an obscurant smoke. The exposure facility was developed through an interagency agreement with the U.S. Army and the U.S. Environmental Protection Agency (EPA). In the field, the obscurant smoke, commonly called fog oil obscurant, is generated by portable units utilizing motor oil. Condensation occurs when the fog oil vapor is diluted by the cool atmosphere resulting in a dense oil fog.

After preliminary work with Oak Ridge National Laboratory (ORNL), the Army decided that using the full scale portable generator for animal exposures was not feasible and a laboratory generator was designed and constructed to generate a diesel fuel obscurant. This generator was made available to the U.S. EPA through a technology transfer from ORNL and was adapted by the U.S. EPA to generate a fog oil obscurant.

An inhalation exposure facility was developed to conduct acute, repeated, and subchronic exposures of rats to the fog oil obscurant. The facility was designed to provide a high level of safety for operating personnel by locating a glass barrier between the operator and the exposure chambers. Four whole-body exposure chambers and two control chambers were installed. Animal quarantine, pre-exposure, post-exposure, and necropsy rooms were located adjacent to the exposure facility. Exposures are conducted from the control room.

The generators supplied by ORNL were modified to eliminate a few minor difficulties discovered during preliminary facility testing. The facility was designed to operate either in a manual or an automated mode. Safety sensors and alarms monitor vital system parameters. In the event of a hardware problem, the safety alarms are activated and the exposure is automatically terminated. Real-time aerosol monitors (RAM-1) continuously analyze the fog oil concentration providing a real-time aerosol mass concentration signal, and a strip chart recorder documents the RAM-1 signal. Filter samples are taken to calibrate the RAM-1 and provide the actual fog oil mass concentration values.

The total quantity of bulk oil required to complete all studies was estimated, obtained, and stored. In order to evaluate the stability of the bulk oil with time, a chemical characterization procedure was developed. The fog oil aerosol or bulk oil samples are analyzed by high performance liquid chromatography. Comparisons will be made between the various peaks, and quality control charts will be developed to monitor any change in stability of the chemical constituents.

Prior to conducting any animal exposures, extensive distribution testing was performed to evaluate the uniformity of the fog oil aerosol throughout the exposure chamber. Distribution testing ensures the test animals are presented with a uniform fog oil aerosol concentration.

The facility was tested and operated for over one year. The exposure facility has generated stable fog oil aerosol concentrations from 0.4 to 10 mg of fog oil per liter of air. The oil aerosol particle size distribution resulted in a mass median aerodynamic diameter of 1.0 to 1.3 μm with a geometric standard deviation of 1.5.

Routine fog oil exposures of rats to a variety of concentrations have been conducted for several months. Exposures will continue for approximately one year according to the experimental protocol.

FOREWORD

Support services for this study were provided by Northrop Services, Inc., under U.S. EPA Contract Number 68-02-2566 from 9/81 through 6/83 and Number 68-02-4032 from 7/83 to present. This work was conducted in response to Section 2 of Technical Directives 4.4-44 (9/81 to 6/83) and 4.1.2 (7/83 to present).

Major contributions were provided by Michael Hiteshew, John Harris, Leon C. Walsh, III, and Margaret Beaman, and editorial assistance was provided by Linda Cooper.

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INTRODUCTION

Visual obscurant smokes are used by the military to conceal personnel, materiel, or installations from direct visual observation. The smoke from a petroleum distillate product is generated by injecting a light lubricating oil into a fog oil generator where it vaporizes and eventually recondenses in the atmosphere. Army personnel may be exposed to this smoke when it is released into the environment in training or combat operations. Since this petroleum smoke is a large area screening obscurant, the duration of exposure may be several hours within a single day, and exposures may be repeated over consecutive days. Evaluation of the potential hazards posed by this smoke to human health is a necessary portion of the data base required to establish comprehensive health criteria for the field use of smokes and obscurants. To perform such health studies, a facility for inhalation exposures of animals was designed, constructed, and tested. This facility is the subject of the report.

INHALATION EXPOSURE SYSTEM DESCRIPTION

An exposure facility designed for animal inhalation exposures and biological testing on exposed animals was designed and constructed by EPA with the support of its on-site contractor. Generators supplied by ORNL were modified to provide stable, drip-free operation.

The smoke exposure facility consists of an exposure control laboratory, exposure room, necropsy room, shower room, and three total-exhaust animal rooms. Figure 1 shows the exposure facility layout. Individual room layouts are shown in Figures 2, 3, 4, and 5. A barrier separates the exposure control laboratory from the exposure room. All air lines and electrical connections for exposure monitors and control devices pass through sealed bulkheads affixed to the barrier wall. The top half of the barrier is constructed with plate glass windows to allow visual inspection of the exposure room. Two double door pass-through windows connect the exposure room to the animal necropsy room. The quarantine and pre-exposure animal holding rooms are separated from the exposure room by an air lock to prevent cross-contamination between rooms or direct contamination from the exposure room. The post-exposure animal room connects directly to the exposure room. All rooms are provided with preconditioned single-pass air with an exchange rate of approximately ten changes per hour.

Conditioned air is supplied to the laboratory area through volume dampers controlled by thermostats in each room. Thus, the air temperature in each laboratory can be controlled within the limits of the building system. Room air is filtered for removal of unwanted gases and particles prior to entry into the exposure chambers.

CHAMBER AIR SYSTEM

Whole body exposures, both exposed and controls, are performed in stainless steel Rochester chambers. The system design allows use of any one or more of

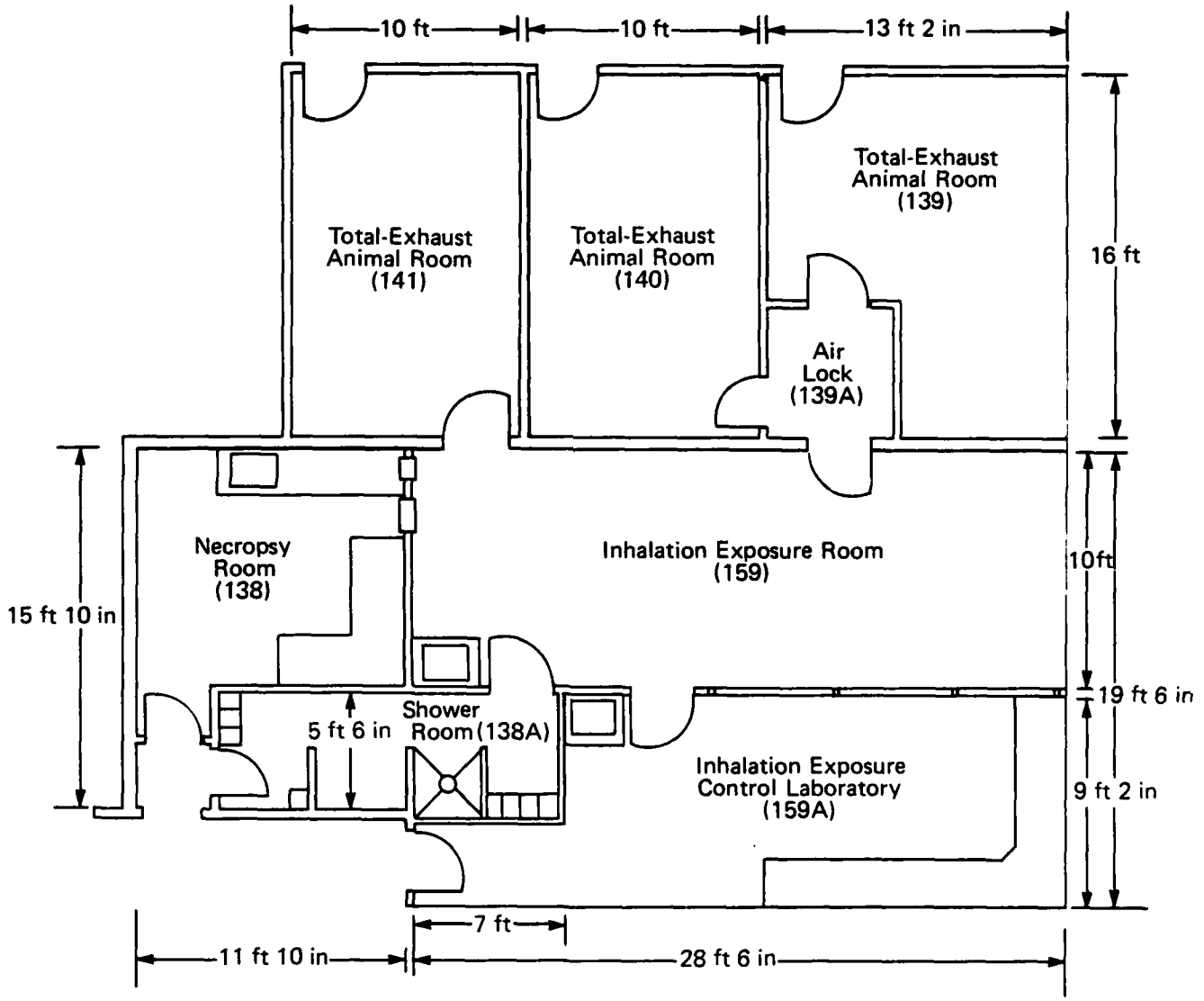


Figure 1. Exposure facility floor plan.

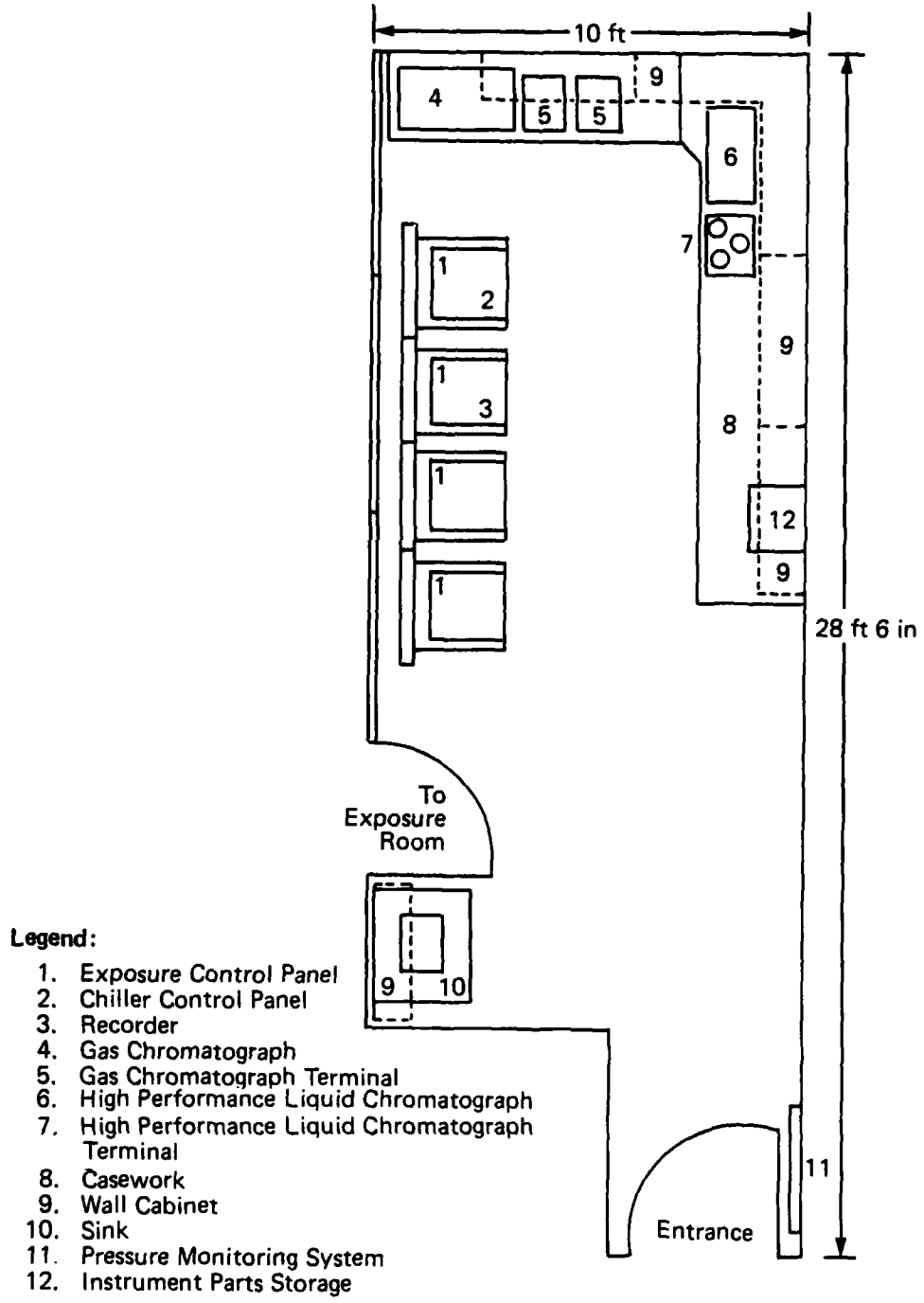


Figure 2. Exposure control laboratory - plan view.

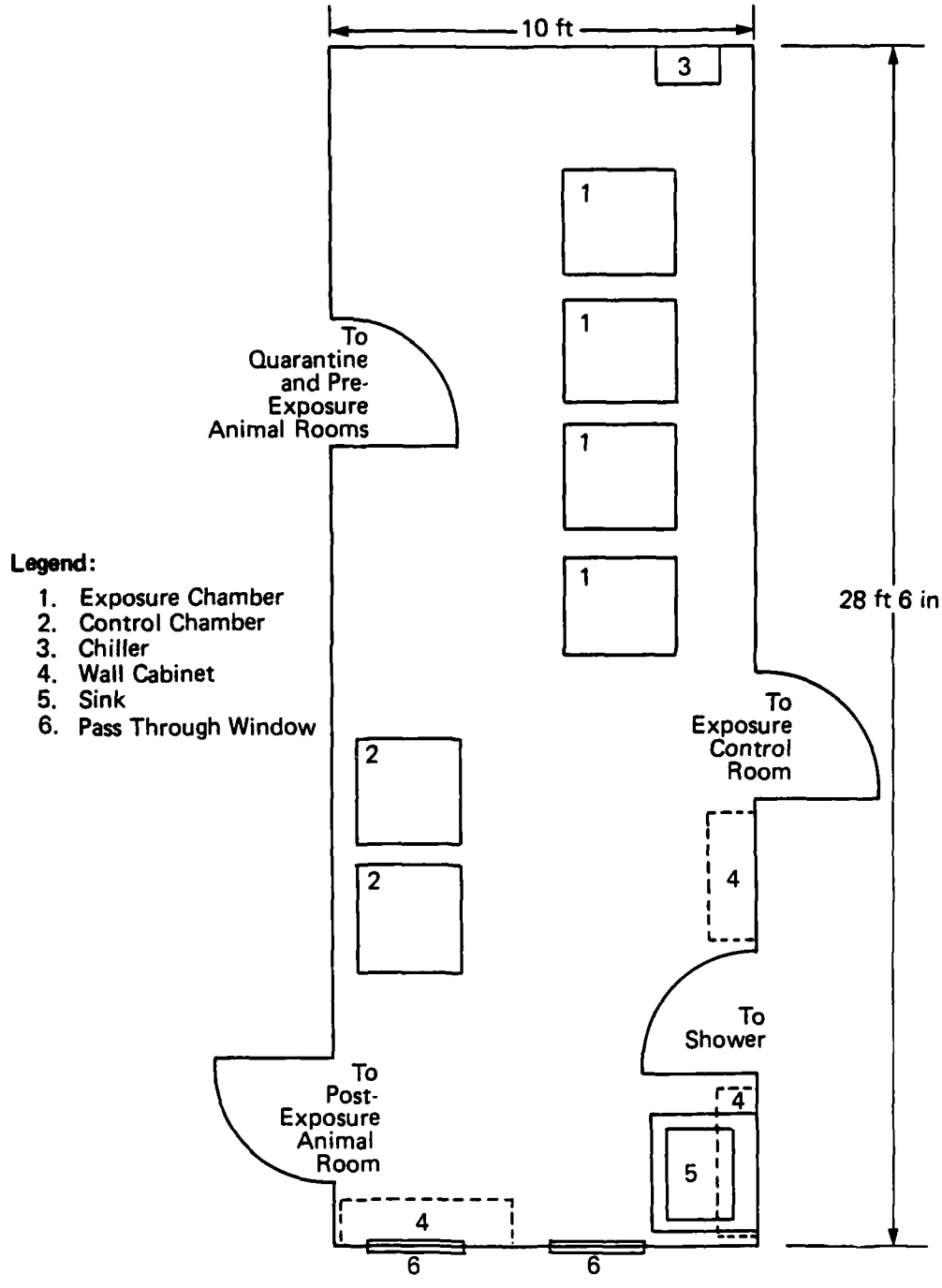


Figure 3. Exposure laboratory - plan view.

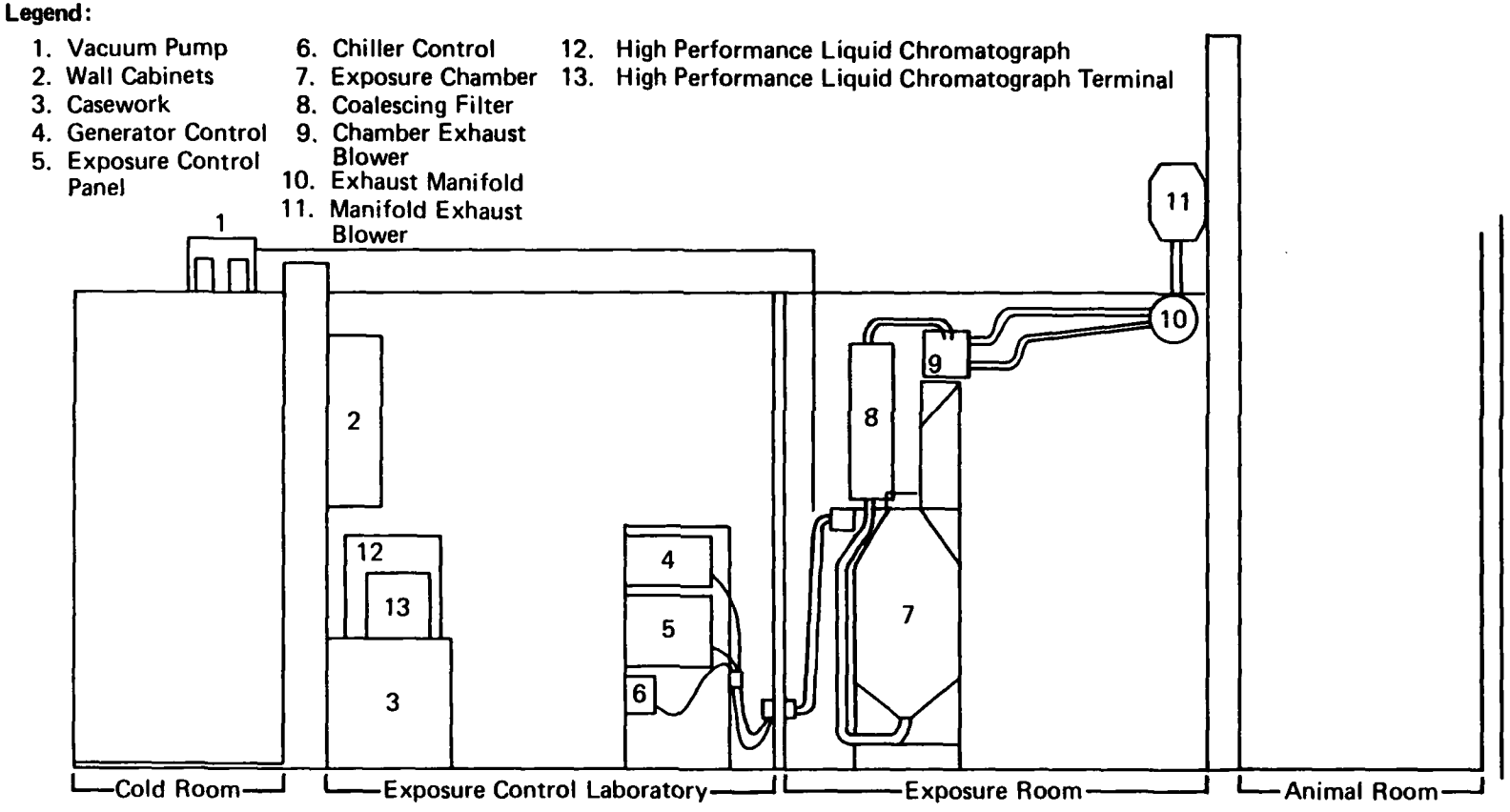


Figure 4. Laboratory - elevation.

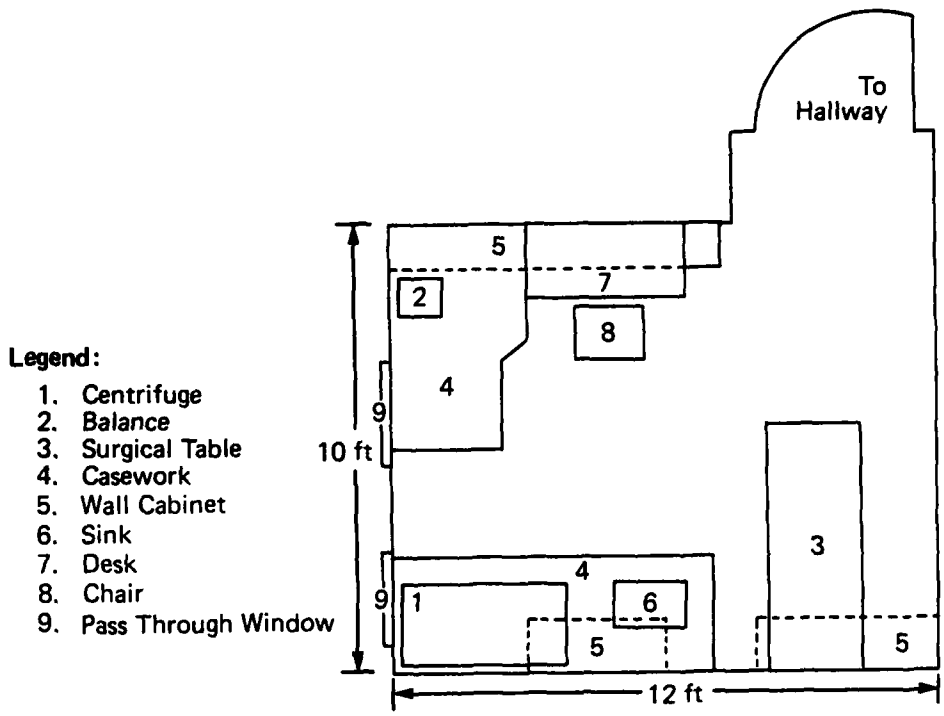


Figure 5. Necropsy laboratory - plan view.

the exposure chambers simultaneously; therefore, a high degree of flexibility in chamber operation is provided. Refer to Figure 6 for a schematic of the chamber air flow. Critical parameters considered in the system include: chamber temperature (74°F ± 4°F desired); flow of air passing through the chamber (approximately 9.4 ft^3/min); velocity of particles in the ducts and chamber (to minimize surface impaction and deposition and ensure adequate mixing); chamber pressure; final removal of aerosol particles from the atmosphere; and disposal of animal waste.

Chamber temperature is maintained at the desired level (74°F ± 4°F) by a cooling unit controlled by an electronic thermostat. Chamber temperature is monitored by a K-type thermocouple wired into the thermostat. A digital volt meter displays actual chamber temperature at the temperature control panel in the exposure control room. A chiller control panel allows the operator to monitor the cooling system performance throughout the course of an experiment. Coolant temperature is monitored at the supply pump and the return side of each inlet air cooling coil. The thermostat controls a three-way solenoid valve, which regulates the flow of coolant to the coil. Coolant flows until the chamber temperature is lowered to the desired level. Relative humidity for the laboratory room is monitored in the building air conditioning return air duct and is normally maintained at 50 percent RH ± 10 percent.

A manually adjustable blower (1/25 horsepower AC/DC adjustable 0 to 75 CFM) (Model 6 SID; Young and Bertke, Cincinnati, OH) pushes air from the exposure room through the inle. air coil and inlet particle and gas filters and into the generator loop. Two inch ID ~'ass tubing is used for the air inlet system. The air is split into two streams before it reaches the generator. This split stream arrangement allows control of the air velocity past the generator while maintaining a constant air flow through the chamber. The generator and bypass air streams are combined, and the aerosol passes through a 0.875-in. orifice plate used to monitor flow and to enhance stream mixing. The aerosol is deflected along the chamber perimeter by a conical shield. Two perforated baffle plates (1/16-in. holes, 10 percent free space) installed in the top pyramid of the chamber complete the mixing process and provide homogeneous chamber aerosol distributions.

The chamber is vented for exhaust and animal waste removal below the bottom cage shelf. Chamber atmosphere is exhausted through 2-in. ID PVC pipe ducting. A coalescing filter (Type R-0780-3T; Balston, Inc., Lexington, MA) removes the oil aerosol particles. Air flow through the coalescing filter is provided by a high pressure blower, which injects the filtered air into a common exhaust manifold. Check valves prevent backflow from the manifold into unused chambers or vacant manifold ports. A light-duty exhaust blower injects manifold air into the room air exhaust system where it passes through high efficiency particulate air (HEPA) filters and charcoal adsorbers prior to being exhausted from the building.

AEROSOL GENERATING AND MONITORING SYSTEM

Generation of the test atmospheres is accomplished by a vaporization/ condensation method. The vaporizing portion of the generation system supplied by ORNL is illustrated in Figure 7. Bulk oil is injected under pressure, using

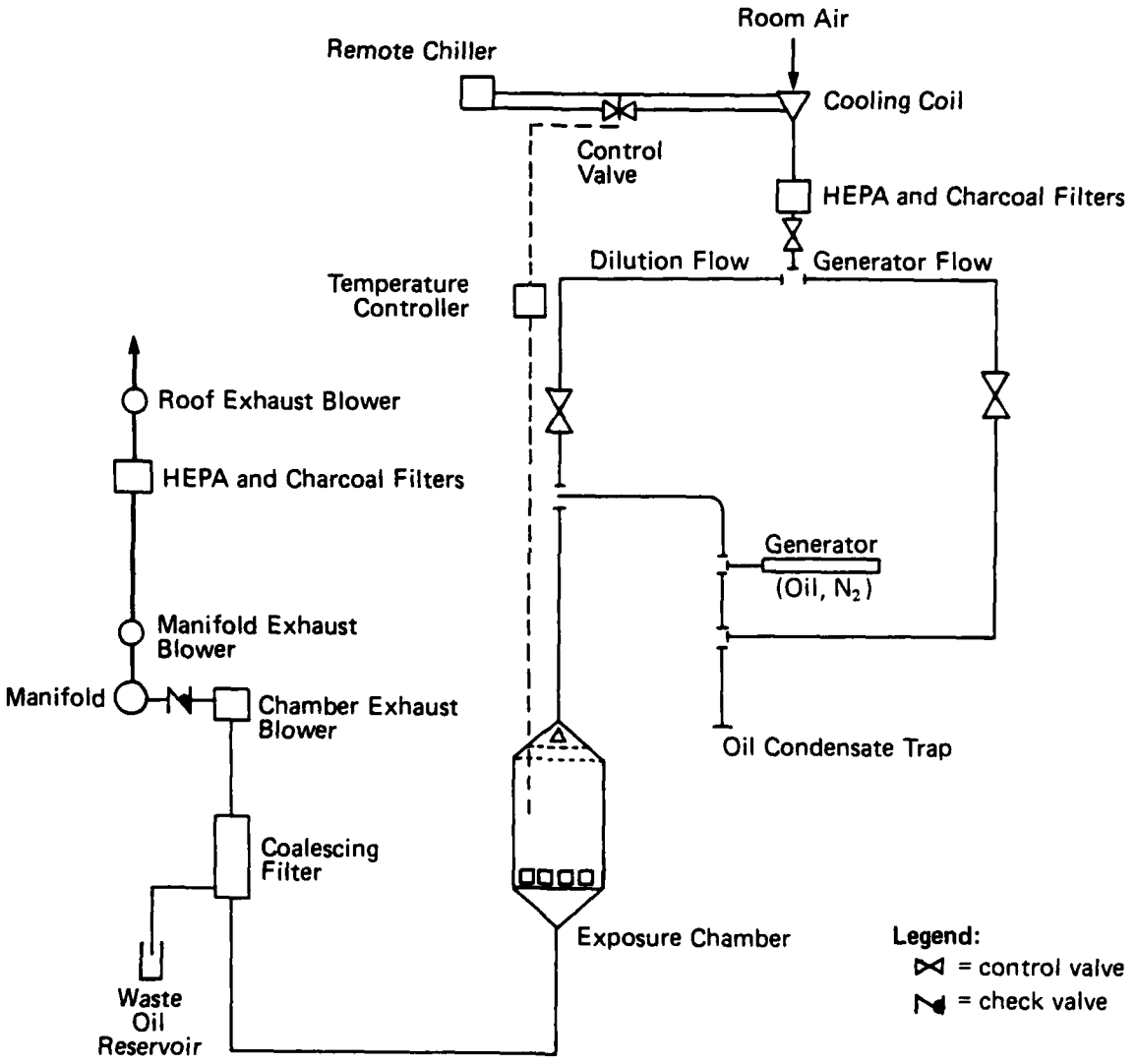
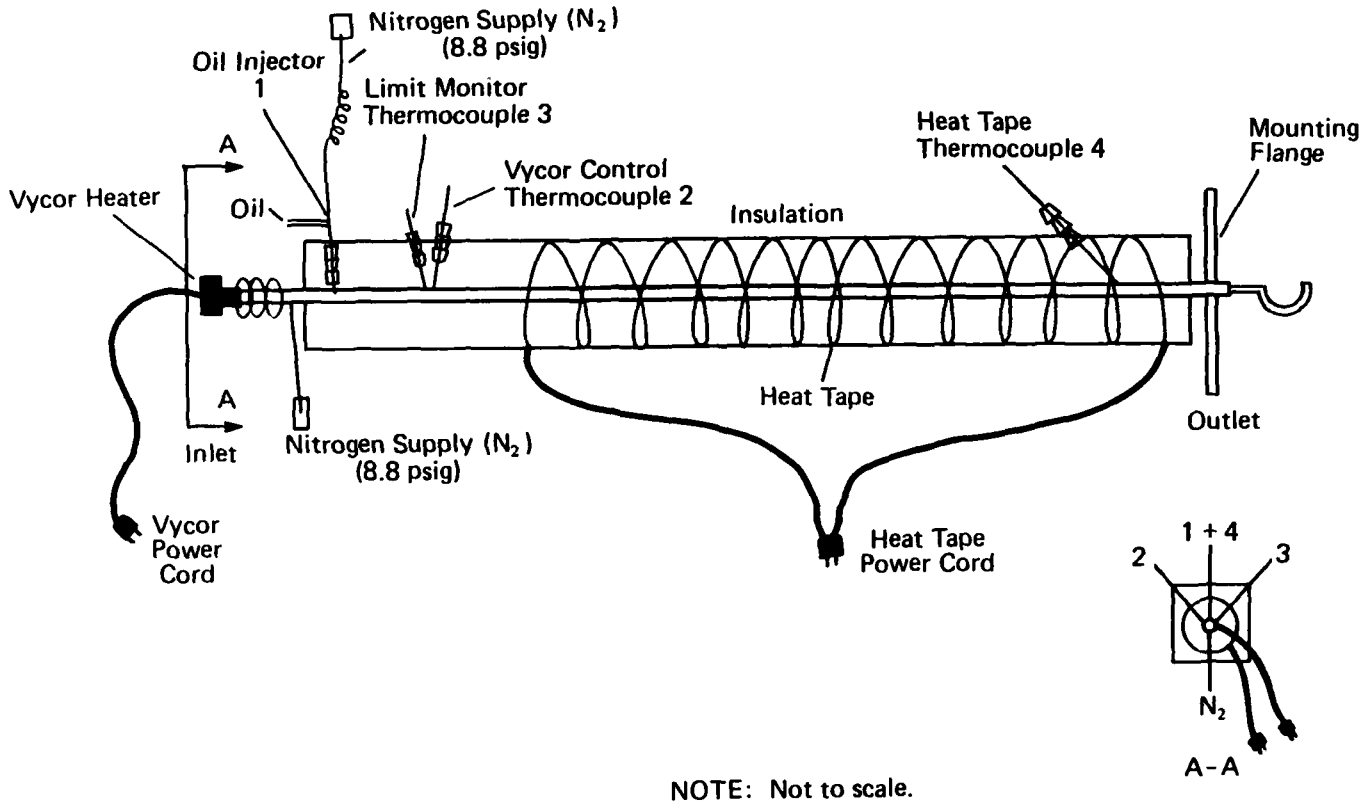


Figure 6. Chamber air flow schematic.



NOTE: Not to scale.

Figure 7. Oak Ridge National Laboratory generator.

nitrogen as a carrier gas, onto a 1,000-W Vycor glass heating element (Model 16790; Corning Glass Works, Corning, NY) in an inert nitrogen atmosphere. The Vycor heater is maintained at 600°C, as established by Holmberg (1), by a dual set point temperature controller (Model 4002; Omega Engineering, Stamford, CT). The adjustable temperature limit setting on the controller is maintained at 700°C which provides an adequate safety margin to prevent damage to the generator. A secondary temperature monitor (Model 4001; Omega Engineering, Stamford, CT) is also set to 700°C to ensure maximum temperature protection for the Vycor portion of the generator. The oil is vaporized in nitrogen to avoid combustion. Premature condensation of the oil vapor/nitrogen mixture is minimized by heat tape wrapped around the generator tube. The heat tape is maintained at 350°C by a dual set point controller which has an upper temperature limit of 425°C.

The oil vapor/nitrogen mixture is injected into the precooled chamber air stream where the oil vapor instantly condenses to form the aerosol droplets.

Part of the total nitrogen (N_2) flow through the generator is diverted through the oil injector to prevent clogging by residual oil. Total nitrogen flow through the generator is held constant at 4.5 Lpm. Vapor concentrations in the N_2 stream flushing the generator are varied in the range of 25 to 630 mg oil/L N_2 to provide fog oil chamber concentrations from 0.4 to 10 mg oil/L air.

Aerosol concentration in the exposure chamber is monitored using RAM-1. Calibration curves comparing the signal with aerosol concentrations determined from filter samples of chamber atmosphere are developed to allow real-time continuous concentration monitoring. Concentration readings from the RAM-1 sensors are continuously recorded on two dual channel strip chart recorders (Model 785V; E and K Scientific Products, Saratoga, CA). Chamber temperature is also displayed, monitored, and recorded for later reference on a 12-channel analog and digital recorder (Dianagraph; Bailey Instruments, Inc., Rockaway, NJ).

The exposure system is monitored and controlled from a remote station in the exposure control laboratory. Refer to Figure 8 for the exposure flow diagram. Magnehelic gauges (Model 2060; Dwyer Instruments, Michigan City, IN) are used for monitoring pressure drop across the coalescing filter (10 to 60 in. H_2O), chamber pressure with respect to room ambient pressure (0.1 to 0.5 in. H_2O), and pressure drop across the chamber flow monitoring orifice. Generator nitrogen flow is monitored and controlled with a mass flow controller (Model FC260; Tylon Corp., Torrance, CA). All parameters, including the liquid oil injection rate, are controlled by manual adjustment of potentiometer circuitry, which is compatible with computer control.

CONTROL AND MONITORING SYSTEM

The System Description

The process control unit is fabricated and assembled by the Adaptive Data Acquisition and Control Corporation (ADAC) and uses the PROSYS I operating system with 256K bytes of memory custom designed for industrial process

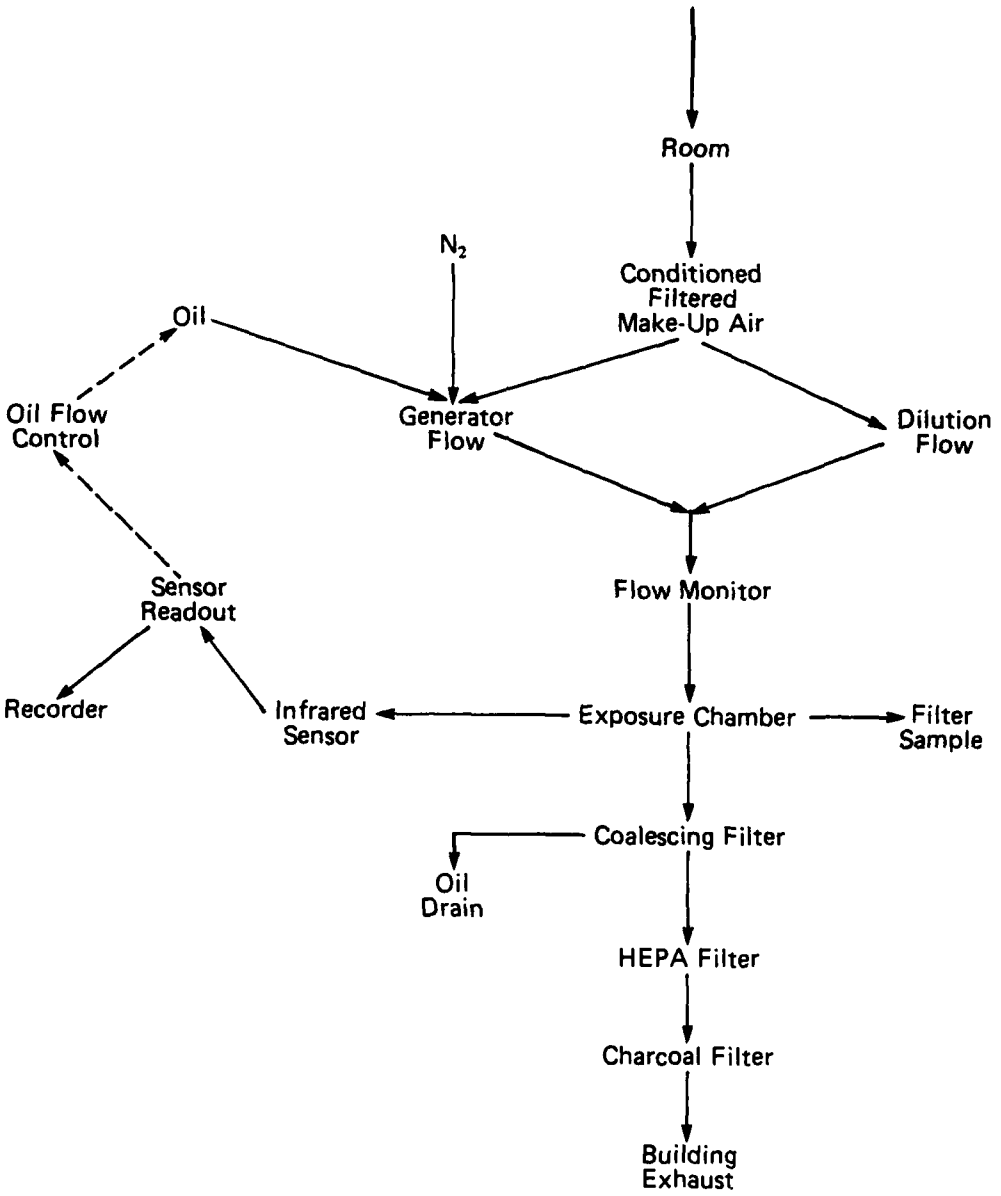


Figure 8. Exposure flow diagram.

control. Peripheral devices include a printer (Model LA120; Digital Equipment Corporation (DEC), Maynard, MA), a terminal/monitor (Lear Siegler Model ADMS; Lear Siegler, Inc., Anaheim, CA) , a floppy disk drive data storage unit (Model RX02; DEC, Maynard, MA), and a video monitor (Sony Trinitron Model CVM 1900; Sony Corporation, Park Ridge, NJ). The computer is interfaced to monitor the following system functions: chamber temperature, Vycor temperature, heat tape temperature, chamber negative pressure, generator nitrogen flow, and RAM-1 concentration sensor for each chamber. The computer system is interfaced to control the exhaust blower speed and oil flow to the generator. Figure 9 illustrates the control system configuration.

Using the PROSYS I measurement and control system with PRO software, the tasks were written such that the subtasks are all initiated by a main task. As each subtask terminates, it sets a flag which signals the main task to deactivate this subtask and activate the next subtask. This approach has allowed modular design of all tasks so only one task is active at any given moment. Thus, there is no competition for the computer's time and resources by the other tasks. This has allowed the tasks to be broken down into manageable units for easy debugging and correction as necessary.

The system of tasks includes the following:

STARTEXP - This is the main task which activates and deactivates all subtasks at the start of an exposure. After activating each subtask, STARTEXP waits for the subtask flag to be set which signals STARTEXP to deactivate the subtasks. This task also initializes all storage arrays to zero before any data are stored. It requests the operator to input the run number.

INIT - The initialization subtask requests the operator to input the start and stop times of exposures from the printer/terminal.

BITS - This subtask activates the high current outputs (HCO's) so the Vycor heaters and heat tapes for all generators can be turned on and off.

BLWRS - Activates the overconcentration controls for all chambers.

HTRS - This subtask resets the chamber controllers, turns on the Vycor and heat tapes for all chambers, and waits until the Vycors are greater than 500°C. The HTRS turns the Vycors off for 30 sec to let them coast up to approximately 600°C and then turns the Vycors back on. Finally HTRS turns all resets off so the controllers can shut the systems off (if heaters exceed specified limits).

OILFLW - OILFLW initializes the oil solenoids, oil pumps, and oil flow, then activates the oil solenoids and oil pumps by increasing the voltages to the relays to ±5 VDC. The OILFLW brings the oil flow voltage up to ±0.515 volts for all chambers sequentially.

MONITOR - This subtask activates another subtask called report, continually checks for end of exposure, and saves final readings of all chamber concentration sensors, and prints final reports on the printer.

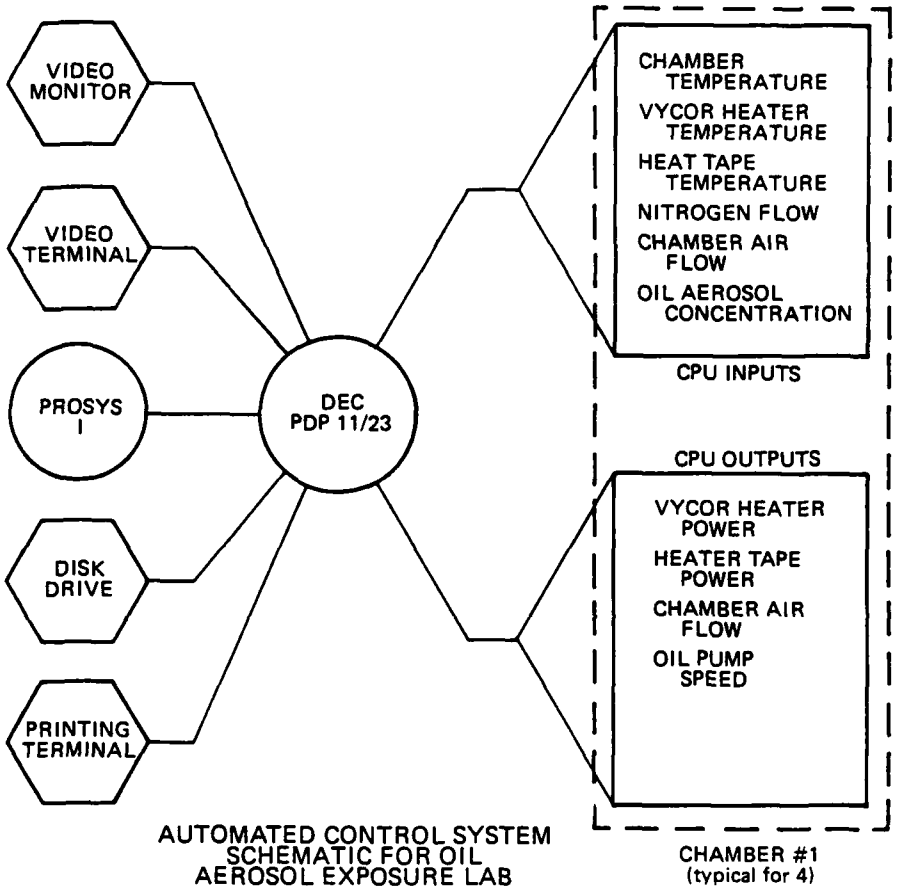


Figure 9. Control system configuration.

REPORT - This subtask prints the concentration readings for all four chambers every 5 min and saves these readings in storage arrays.

SHUTDOWN - This subtask deactivates oil flow, oil pumps, and solenoids, shuts off heaters, waits until Vycor temperatures are less than 200°C, and deactivates overconcentrations for all chambers.

DWRT12 - This subtask writes the concentration arrays for Chambers 1 and 2 into disk files.

DWRT34 - DWRT34 writes the concentration arrays for Chambers 3 and 4 into disk files.

SAFETY SYSTEMS

The safety of operating personnel, equipment protection, and integrity of test data were a major concern throughout the development of the aerosol exposure system. A preliminary systems analysis was performed to ensure adequate project planning. This analysis involved general discussions with Robert Holmberg and associates of ORNL, as well as intergroup review of project plans. The existing system resulted from the various ideas discussed during planning.

The safety systems guard against laboratory exhaust system failure, chamber exhaust failure, and generator overtemperature. Photohelic gauges monitor room and chamber exhaust conditions, and pressure transducers monitor chamber pressure. Vycor heating elements are protected by an overtemperature safety device in the primary controller. Because overtemperature in the heating elements poses a serious safety threat, an independent temperature monitor is used as a secondary safety device. If system failures occur, such as loss of exhaust blower and/or chamber negative pressure, or overtemperature of the generator heating element, the safety system deactivates the generator heaters and closes the solenoid valve on the oil supply line. These system failures are signaled by audible and visual alarms. Once the safety system has responded to a system failure, the generation system will not restart until it is reset manually.

STANDARD OPERATING PROCEDURES

The standard operating procedures are presented in three major sections. Each section is independent and can be used without reference to the other sections. The first two sections document the procedure necessary to prepare the oil aerosol exposure system for operation and explain exposure system operation. The last two sections detail the procedures needed to perform the animal exposures. Specific operating procedures for the exposure studies and facility maintenance are in Appendix C.

For the standard operating procedures, it is assumed that the operator is familiar with the various subsystems of the oil aerosol facility, as well as the cooperative services offered by other building personnel. Subsystem

descriptions are outlined in the previous section. Cooperative services are coordinated by the Inhalation Facility building manager. New personnel must be carefully trained by experienced operations personnel.

CHAMBER SET-UP: PRE-EXPOSURE PROCEDURE

This procedure assumes that the inlet and outlet plumbing, generator, oil injector, and temperature controls are assembled and mounted on the chamber. Additionally, the operator should be familiar with all aspects of the exposure system. Exposure parameters, such as pollutant concentration, length of exposure, and number and sex of animals, are specified on the exposure request form submitted by the principal investigator. System operating parameters (e.g., generator temperature, nitrogen flow, chamber air flow, and recorder setup) are determined by preliminary system testing and/or experimental protocol.

The following pre-exposure checks must be performed for proper chamber setup:

1. Check the chamber and plumbing to ensure tight joints, capped ports, and adequate door seals.

2. Refer to the procedure for aerosol concentration determination (Appendix C) and install and calibrate the RAM-1 sensor.

3. Calibrate the orifice for the filter sample train.

4. Set up the air chiller inlet.

5. Empty the waste oil reservoirs.

6. Fill the supply oil reservoir and check the pump tubing.

7. Set up the recorder.

8. Energize the temperature, chiller, and exposure control panels.

EXPOSURE OPERATION

This procedure assumes that the operator is familiar with the operation of the exposure facility and all associated equipment and instrumentation. Refer to Appendix C for detailed procedures. Before beginning the oil aerosol exposure, the pre-exposure procedure (preceding section) should be performed.

The oil aerosol concentration and duration of exposure are listed on the exposure request form along with the number, species, and sex of the animals used. One, two, three, or four chambers may be brought on line simultaneously. Use the following procedure:

Daily Exposure Operation - Manual

1. Energize the manifold exhaust blower.

2. Energize the chamber exhaust blower.

3. Energize the chamber inlet blower and piston vacuum pump.

4. Adjust the chamber air flow and pressure valves.

5. Reset the malfunction alarm.

6. Check the nitrogen supply manifold pressures (55 psig main/45 psig backup).

7. Open the nitrogen main shut-off valve in the laboratory and set the regulator at 8.8 psig.

8. Start the recorder (RAM-1 output) and Dianagraph (chamber temperatures).

9. Energize and reset the nitrogen solenoid valve. Adjust the nitrogen flow to the protocol level.

10. Verify the heater and limit control settings (Vycor: set 600°C, limit 700°C; heat tape: set 350°C, limit 425°C).

11. Energize the heaters. Monitor the Vycor temperature until it reaches 500°C; then switch off the Vycor and allow it to coast upward until a temperature peak is reached (usually around 590°C).

12. Turn on the Vycor and monitor the controls until all heaters have stabilized at their set points. Refer to the temperature controller manual for drift correction procedures, if required.

13. Verify that the settings of the pump power and pump mounted speed control are correct.

14. Energize and reset the oil solenoid. Energize and reset the oil pump power.

15. The aerosol concentration is adjusted by the oil pump speed control. Increase the speed setting to increase the oil aerosol concentration. Visually verify the aerosol generation. Inspect the generation system for leaks.

16. Monitor the chamber air flow and temperature. Adjust as required.

17. Fifteen to twenty minutes are required for the concentration to stabilize. At this point, obtain a quality assurance filter sample. Refer to the aerosol concentration determination procedure (Appendix C). Compare the gravimetric measurement with the desired concentration and adjust the pump speed as required. Note the RAM-1 sensor readout at the desired concentration.

18. Monitor the chamber air flow and aerosol concentration (RAM-1 recorder output) and adjust each as required. Refer to the system quality assurance document for gravimetric sampling frequency.

19. The exposure duration is listed on the exposure request form. To terminate aerosol generation, turn off the oil pump power.

20. Wait 1 min and switch off the oil solenoid and de-energize the malfunction alarm, Vycor, and tape heaters.

21. Monitor the RAM-1 recorder output and when the aerosol has been purged from the chamber, de-energize the piston vacuum pump.

22. Continue to monitor the entire system until the Vycor and tape heater temperatures are below 200°C. At this point, the nitrogen flow may be switched off at the main shut-off valve and solenoid valve.

23. De-energize chamber inlet, chamber exhaust, and manifold exhaust blowers.

24. Shut down the recorder and Dianagraph.

Exposure Operation - Automated

Initial run for the day

1. Turn breaker on in back of computer.

2. Turn power switch on in back of terminal.

3. Turn computer on by pushing in DC PWR button on front of top computer unit.

4. When cursor appears on monitor screen enter LO1; and wait for the word DONE to appear on screen.

5. Enter R; and wait until all printing has been completed on screen.

6. Enter the following:

   CHG: DAY, x; Any positive value allows the system to operate.
   CHG: HOUR, xx; 2 digits representing current hour;
   CHG: MIN, xx; 2 digits representing current minutes;
   CHG: SEC, xx; 2 digits representing current seconds at time semicolon key is depressed.

7. Start computer control of exposure by entering RUN: STARTEXP;

8. The system will now request a run number on the LA120 terminal printer: Enter the run number (numbered consecutively during each month) followed by a semicolon character (;) on the terminal.

9. The system will now request start and stop times of the exposure on the terminal. Enter these as requested. When start and stop times have been entered the computer will perform all functions listed under manual operation items 10 to 23.

Second run of the day

Enter the following:

RUN: STARTEXP; and repeat process of entering start time and end time.

POST-EXPOSURE PROCEDURE

This procedure assumes that the operator is familiar with the system instrumentation, animal handling procedures, and operating protocol. (See Appendix C for detailed procedures).

Use the following procedure after the exposure testing:

1. Check the signal from the RAM-1 sensors to ensure that the aerosol is out of the chamber.

2. After the N_2 has been off a minumum of 15 min, remove the animals from the chamber and transfer them to the premarked holding cages. All post-exposure animals are held for observation according to the experimental protocol.

3. Complete the exposure record form for each test chamber used and file the original. File the original recorder tracing.

4. Forward one copy of each of the exposure record forms (Figure 10) to the exposure requester. File all exposure data, exposure record forms, recorder tracings, and computer output in the cabinet located in Room 102 of the Inhalation Facility building.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF FOG OIL

Instrument Preparation

Operating instructions for the chromatograph are given in the Spectra-Physics "SP8700 Solvent Delivery System Operating and Service Manual." The chromatograph is prepared for fog oil analysis using the following procedure:

1. Fill solvent reservoir "A" with spectral grade hexane.

2. Fill solvent reservoir "B" with spectral grade methylene chloride.

3. Allow the solvents to helium degas for approximately 5 min prior to solvent flow initiation. (Section 1.3.3, operating manual).

4. Establish the following gradient program in the chromatograph microprocessor:

Date: _____

Fog Oil Exposure Record

Date of exposure: _____

Investigator: _____

Type of animals: _____

Number of exposed animals: M _____ F _____ Chamber # _____

Number of control animals: M _____ F _____ Chamber # _____

Length of exposure: Start _____ Finish _____ Total Length: _____

Concentration desired: _____ Actual: Mean _____ Std. Dev. _____

Temperature of chamber: _____ Chamber flow: _____

Chamber negative pressure: _____

Vycor temperature: _____

Heat tape temperature: _____

N_2 pressure: _____ N_2 flow: _____

Oil flow: _____

Operators: _____

Filter Sample Data

Time	Sample # Time	Orifice Flow (LPM)	Sample Time (Min)	Sample Weight (mg)	Concentration (mg/L)
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	11				
	12				
	13				
	14				
	15				

Figure 10. Fog oil exposure record form.

Time (min)	% A	% B	Flow (mL/min)
0.0	100	0	0.75
15.0	100	0	0.75
30.0	50	50	0.75
45.0	50	50	0.75
55.0	100	0	0.75

5. Set the recorder chart speed at 0.2 in. per min.

6. Set the UV detector on 0.08 absorbance units.

7. Prior to sample injection, allow the chromatograph to operate using 100 percent hexane at a flow of 0.75 mL/min until a stable baseline is achieved.

Sample Preparation

Bulk oil analysis

1. Place approximately 30 mg of oil into a 25.0 mL volumetric flask.

2. Dilute to 25.0 mL solution with 100 percent spectral grade hexane.

Collected Aerosol Analysis

1. Collect approximately 30 mg of fog oil aerosol on a glass fiber filter pad.

2. Cut the filter pad into 1/8-in. wide strips.

3. Place the filter pad strips into a 25.0 mL volumetric flask.

4. Add 100 percent spectral grade hexane to achieve a total volume of 25.0 mL. Invert. Let stand at room temperature for 1 hour before sampling.

Sample Analysis

1. Fill the 10 μL sample loop with the sample solution.

2. Place the injector in the Inject position.

3. Indicate the injection on the chromatogram with the event marker.

4. Initiate the gradient program.

5. Return the injector to the fill position.

6. After the analysis is complete, allow the chromatograph to operate using 100 percent hexane for $\simeq$ 10 min to clear any residual methylene chloride from the solvent delivery system.

Chromatogram Analysis

1. Construct a hexane baseline (NOTE: Hexane baseline is simply an extension of baseline prior to sample injection).

2. Measure peak heights A through L (Peak height is the distance in millimeters from top of peak to the hexane baseline).

3. Normalize peak heights to sample mass by dividing peak heights A through L by sample mass in milligrams.

RESULTS AND DISCUSSION

OIL AEROSOL GENERATION SYSTEM

The oil aerosol generation system used in this study is essentially the same as that reported by Holmberg (2). Changes in the oil injection and chamber air flow systems were made to improve generator performance for the fog oil.

With the unmodified generator, the formation of a fused oil material at the tip of the injector rapidly plugged the injector and prevented further generation of aerosol. Oil flow rates were sufficiently small to allow fusing to occur as a result of heat transfer from the Vycor heating element. To eliminate this problem, approximately 200 cm^3/min of the total nitrogen flow through the generator was diverted through the oil injector. No further problems with blockage occurred, and the overall operation of the generator was improved.

Using the unmodified generator air flow system, significant amounts of liquid oil were gradually transported into the chamber. Oil accumulation inside of the chamber adversely affected the operation of the diffusion plates, allowed oil to drip onto the animals, and occasionally interfered with the infrared sensor operation. The problem was solved by splitting the chamber air stream into two streams and adding a liquid oil trap beneath the oil vapor injection port. The modification significantly reduced air velocity past the oil vapor injection port, thus allowing the liquid oil to accumulate and drip into the oil trap.

The generation system used in this study is capable of providing a stable aerosol concentration in the range of 0.4 to 10 mg of oil per liter of chamber air. The lower limit is imposed by the liquid oil pump and could be lowered by implementation of a syringe pumping system. Exposures performed in the 0.3 to 10 mg/L range exhibit concentration deviations of less than 10 percent ([standard deviation/actual mean] x 100).

AEROSOL MONITORING

Aerosol concentrations were initially monitored using Gayle/ORNL (2) continuous particle sensors and filter sample analysis. Output from the

Gayle/ORNL sensors was recorded on a continuous recording device and stored for future reference.

Filter sample mass collections are determined gravimetrically. Mass determinations by gas chromatography were attempted, but deviations caused by extraction and sample injection error rendered that method less effective than the gravimetric procedure.

One problem that became evident while conducting exposures was the drift in the Gayle/ORNL particle sensors. Calibration curves were constructed for each infrared sensor by plotting sensor readout versus filter determinations in a range of ±10 percent of the desired concentration. During actual exposures, the calibration curves were not usable because ambient light variations created by the presence of animals and caging significantly changed sensor output, and formation of an oil film on the sensor window significantly altered sensor output as a function of exposure time. Both factors contribute variability in a random fashion and cannot be compensated for during development of the calibration curves. To improve this condition, the infrared sensors were shielded and removed from direct contact with the oil aerosol by mounting each sensor in a small diameter tube attached perpendicularly to a flat black plastic cylinder 6 in. long and 1 in. in diameter. The cylinder was mounted vertically in the chamber to avoid affecting air flow patterns. The sensor was recessed into the small tube to prevent direct contact with the oil aerosol and eliminate ambient light. This modification improved sensor stability but still was not adequate for system control.

In a continuing effort to obtain a real-time aerosol mass monitor, a RAM-1 aerosol mass monitor (GCA/Environmental Instruments Model RAM-1; GCA Environmental Instruments, Bedford, MA) was connected to the chamber. Since the chamber aerosol concentration exceeded the maximum concentration limit of the RAM-1, adjustment of the RAM-1 electronics was attempted, but this created linearity problems. The second choice was to build a dilutor, at approximately a 10 to 1 dilution, to reduce the aerosol concentration to within the range of the RAM-1. This system was set up, tested, and found to operate satisfactorily. Three additional dilutors were constructed and attached to the chambers and are providing real-time aerosol mass concentration data to assist in control of the chamber.

Particle size studies are performed weekly using a 7-stage cascade impactor (Andersen Model 20-830X; Andersen Samplers, Inc., Atlanta, GA). Mass loadings of each stage are determined gravimetrically resulting in a mass median aerodynamic diameter (MMAD) of 1.0 to 1.3 μm with a geometric standard deviation of 1.5.

A complete concentration distribution analysis was performed in all four exposure chambers. The preliminary study indicated that significant concentration differences existed between various points within the chamber. It was determined that preferential flow around the diffusion plates created the concentration differences. The problem was corrected by resealing the diffusion plates. Statistical analysis of the final distribution data is included in Appendix D.

CHEMICAL ANALYSIS

The objective of the chemical analysis protocol is to monitor the chemical consistency of the fog oil exposure atmospheres. The protocol is intended to signal the occurrence of major chemical changes in the fog oil aerosol which result from changes in generation temperatures, oxidation of the oil during some phase of aerosol generation, or chemical variations in the liquid oil used for aerosol generation. Once a major chemical change has been observed, investigators can analyze the effects on the study and initiate appropriate corrective action.

High performance liquid chromatography (HPLC) rapidly and effectively separates and quantitates the various compound classes found in the oil. HPLC coupled with UV detection provides a consistent profile of the unsaturated aliphatics, single and multiple ring aromatics, and semipolar (oxygen containing) compounds. Chemical shifts can be detected from the HPLC profile as changes in peak area (height) of the various compound class. Statistical analysis of the peaks given by the HPLC profile provides a reliable means to monitor chemical changes in the exposure atmospheres.

The example HPLC chromatogram (Figure 11) is a typical profile of collected oil aerosol. Bulk oil used for aerosol generation has a similar profile. Peaks labeled A through L are always present in the profile and are used in the quantitation of the oil sample. Peak heights A through L are collected and each peak normalized to the sum of the peak heights A through L.

A control chart is constructed from statistical analysis of the normalized data from 37 chromatograms. Normalized data obtained from subsequent exposures will be compared to the control chart ensuring that chemical consistency is maintained.

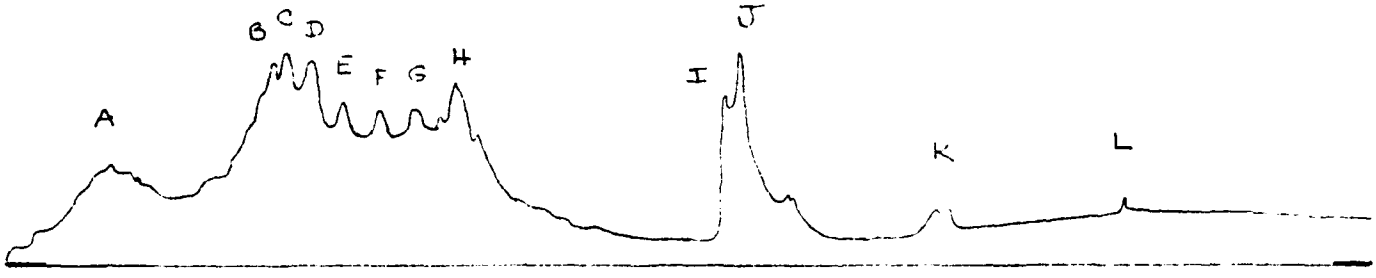


Figure 11. HPLC chromatogram of fog oil.

LITERATURE CITED

1. Holmberg, R.W., Moneyhun, J.H., and Dalbey, W.E. 1981. An exposure system for toxicological studies of concentrated oil aerosols. In B.K.J. Leong, ed. Proc. Inhalation Toxicology Technol. Symp. Ann Arbor, MI: Ann Arbor Science Publishers, Inc.

2. Gayle, T.M., Higgins, C.E., and Stokely, J.R. 1979. Sensor for Detection of Tobacco Smoke Particulate Matter in Inhalation Exposure Systems. ORNL-5424. Oak Ridge National Laboratory, Oak Ridge, TN.

3. Selgrade, M.J. 1983. Appendix B. In Inhalation Toxicology of Fog Oil Obscurant, Bimonthly Progress Report 10, Mar.-Apr. Toxicology Branch, Inhalation Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC.

APPENDIX A

OIL IDENTIFICATION

The military identification of the bulk oil used in the animal inhalation exposure facility described in this report is listed below.

Mil-F-12070A AM.1
Type SGF-2
9150-00-261-7895
2/81 Batch 574
DLA-600-81-0-0689
Manuf. Date: 2/81
Test Date: 2/82

Purchased from Phipps Product Corporation

Boston, MA 02110
phone: (617)542-7341

APPENDIX B

FLOWCHARTS FOR AUTOMATED CONTROL SYSTEM

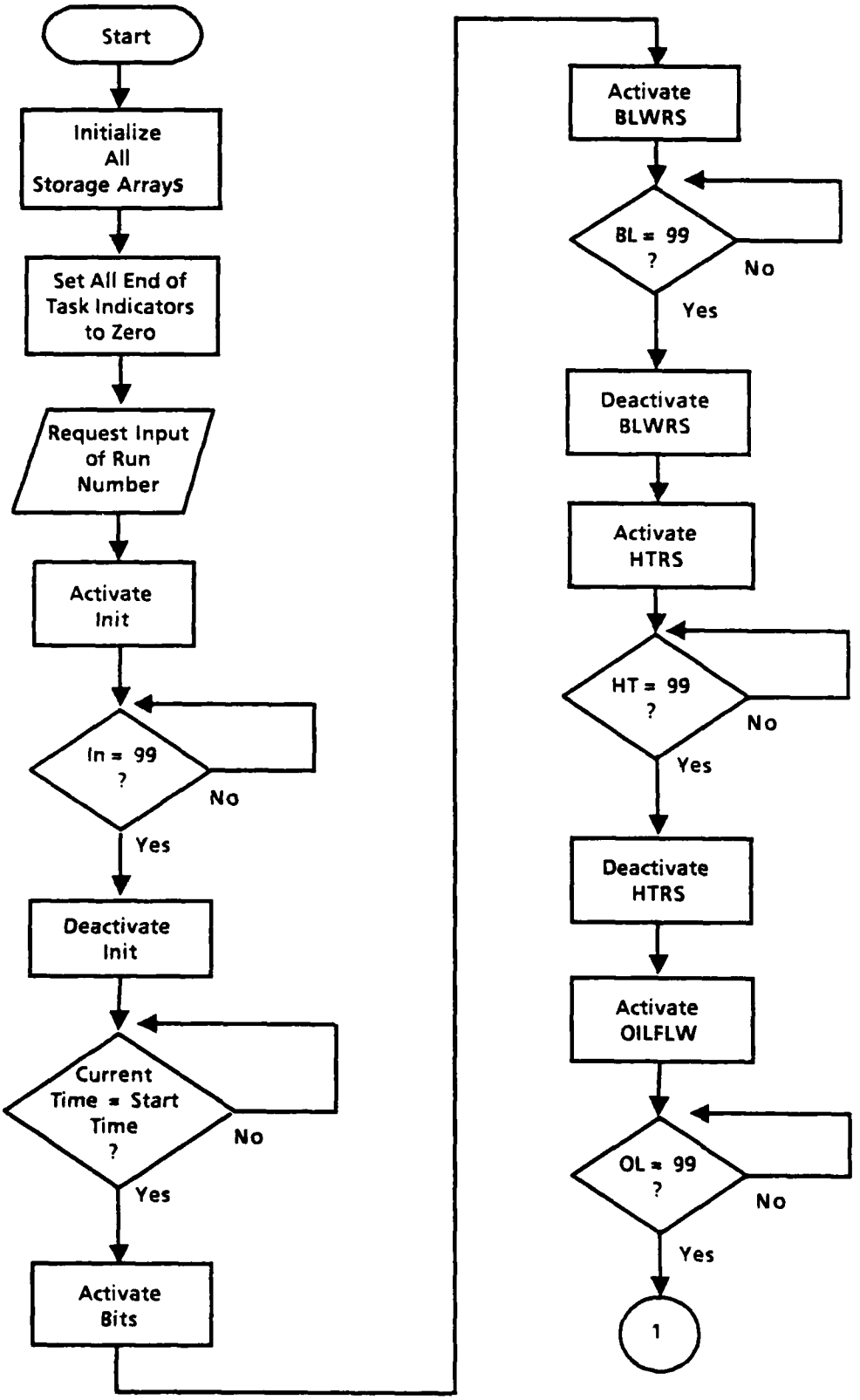


Figure B-1. Main task - STARTEXP.

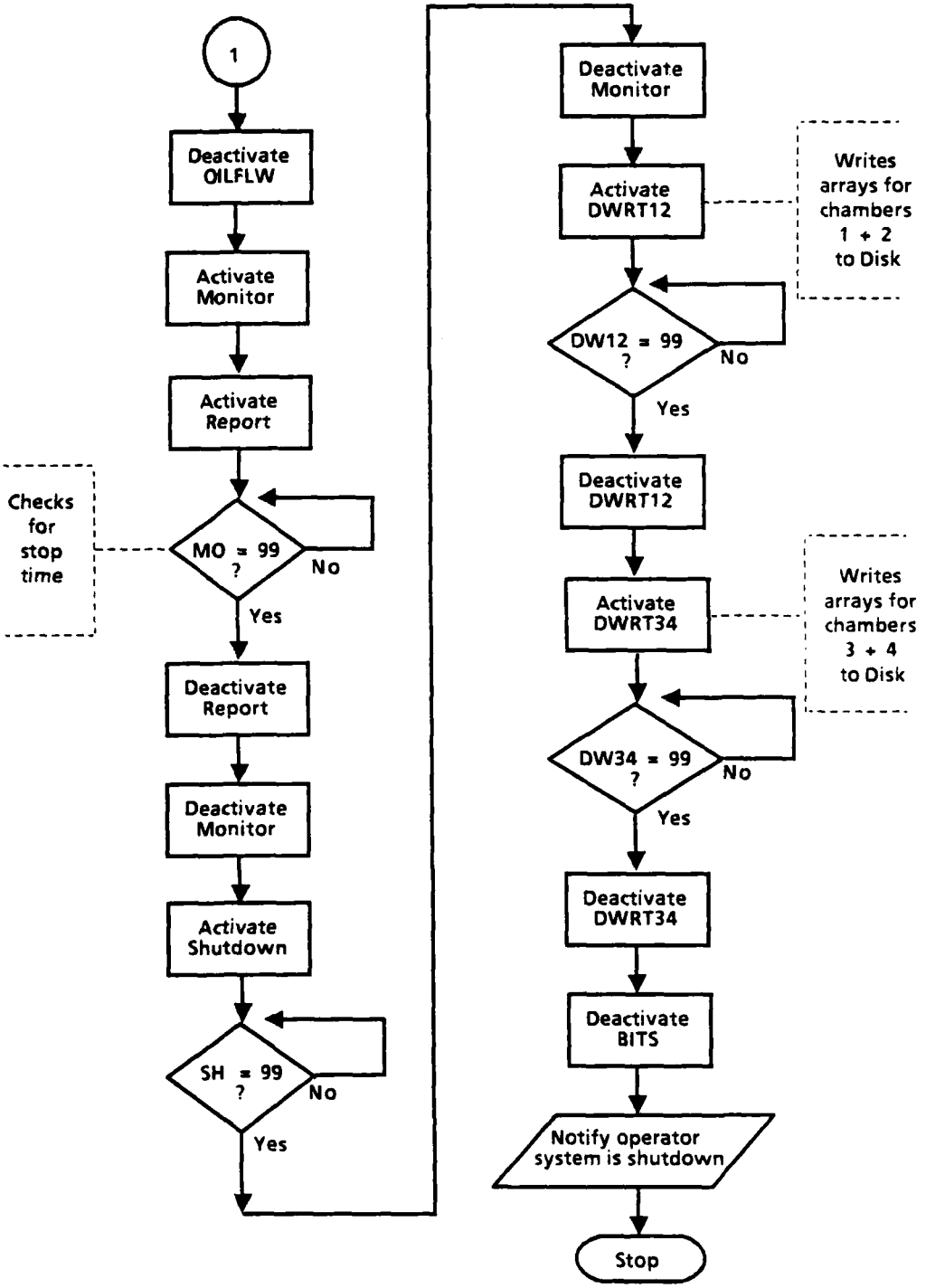


Figure B-1. Continued.

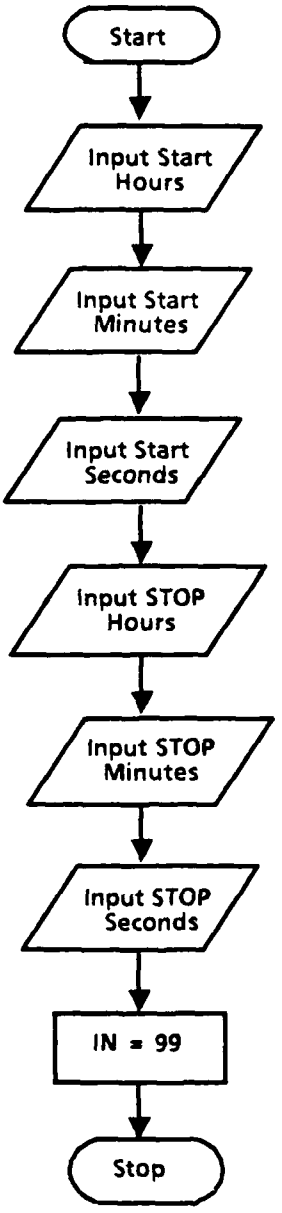


Figure B-2. Subtask - INIT.

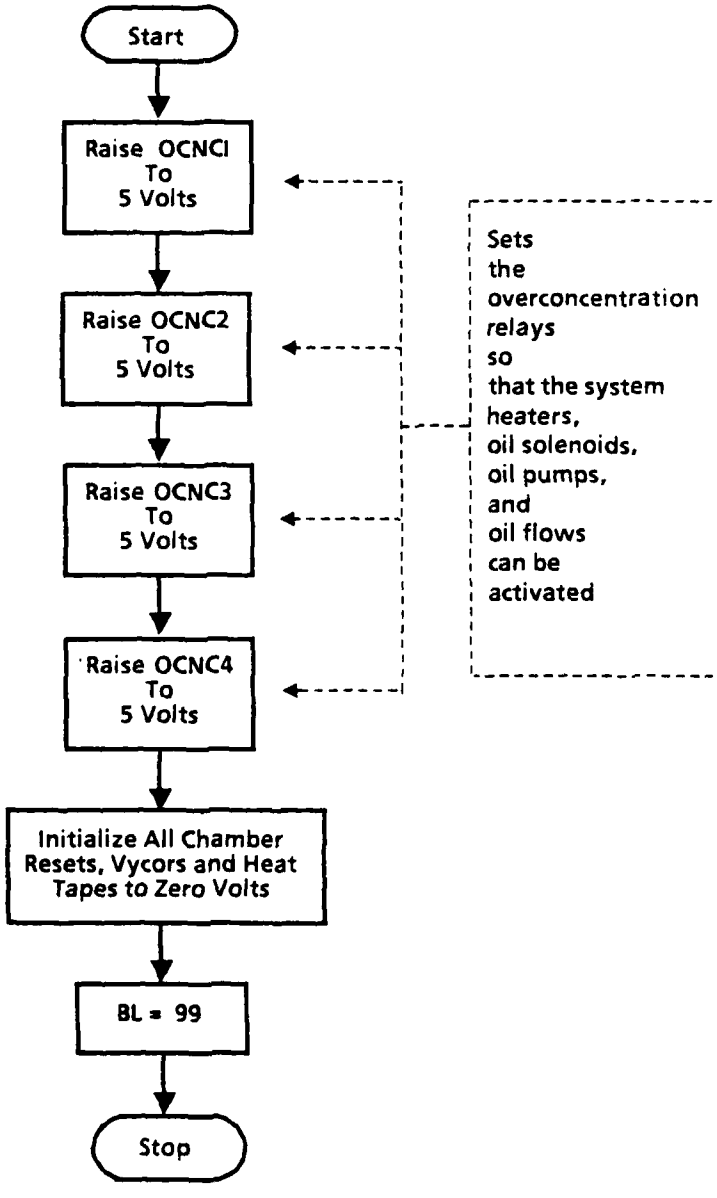


Figure B-3. Subtask - BLWRS.

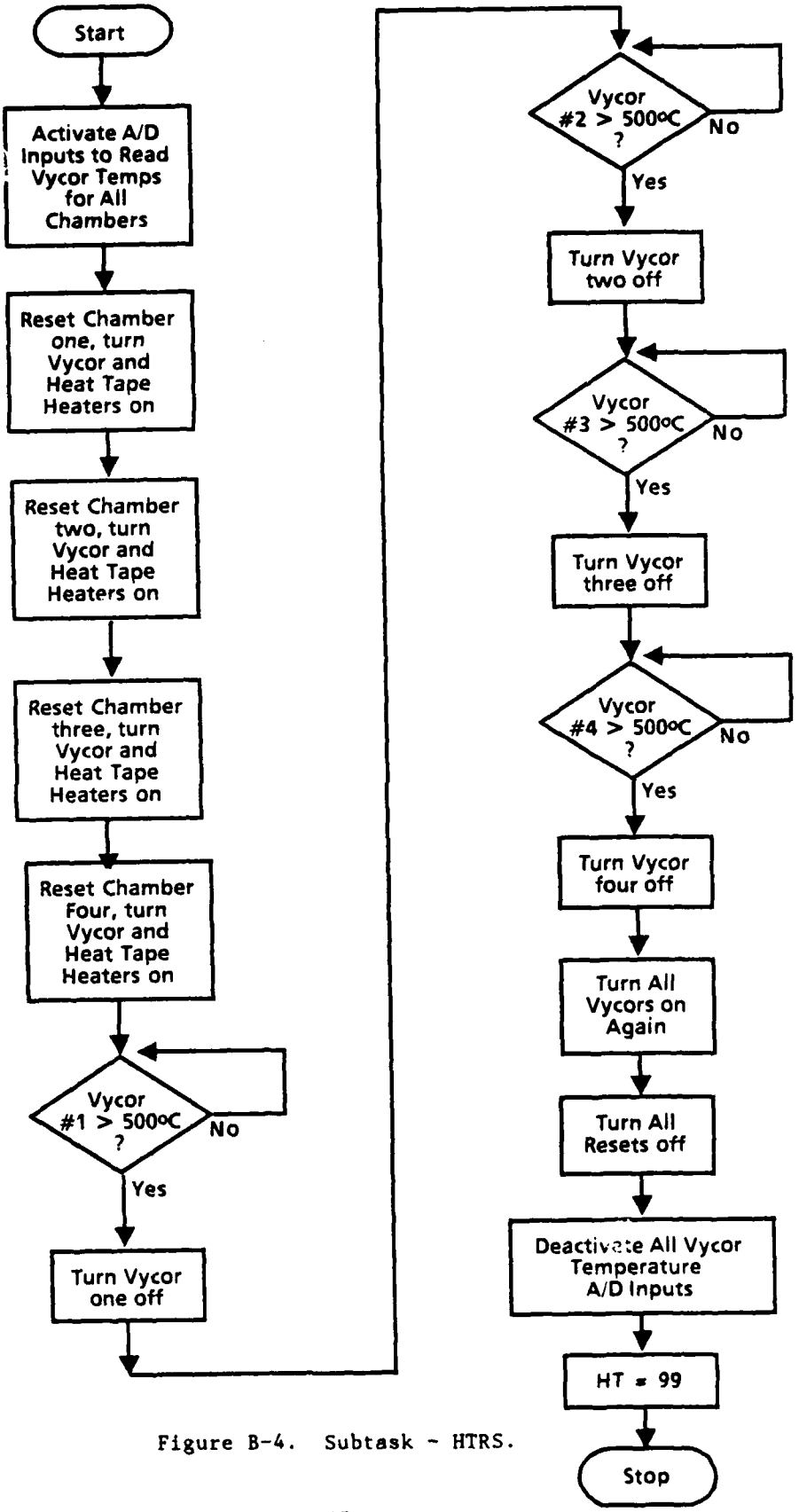


Figure B-4. Subtask - HTRS.

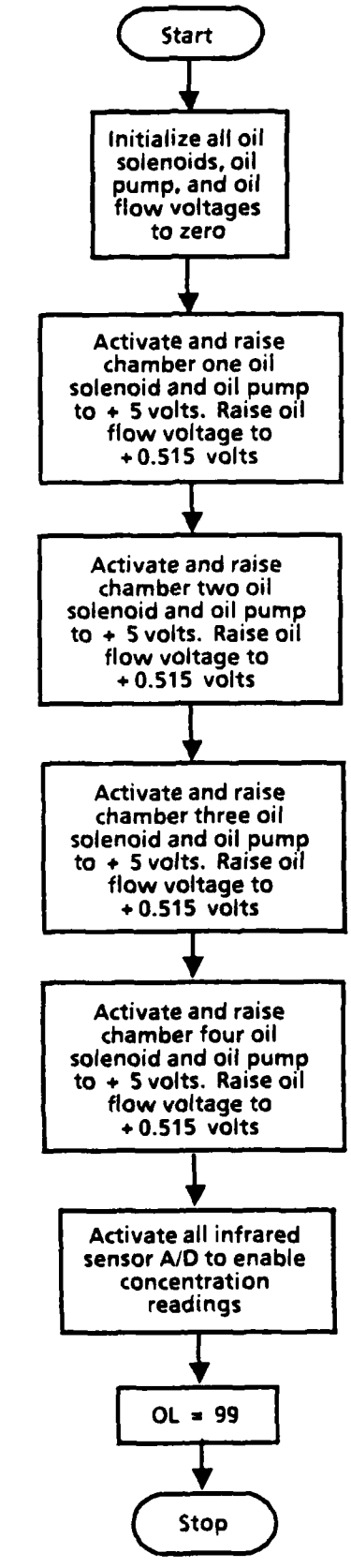


Figure B-5. Subtask - OILFLW.

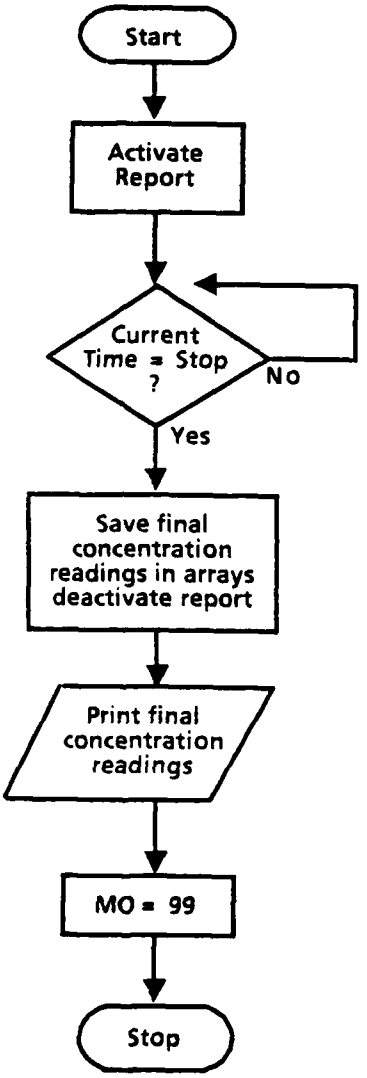


Figure B-6. Subtask - MONITOR.

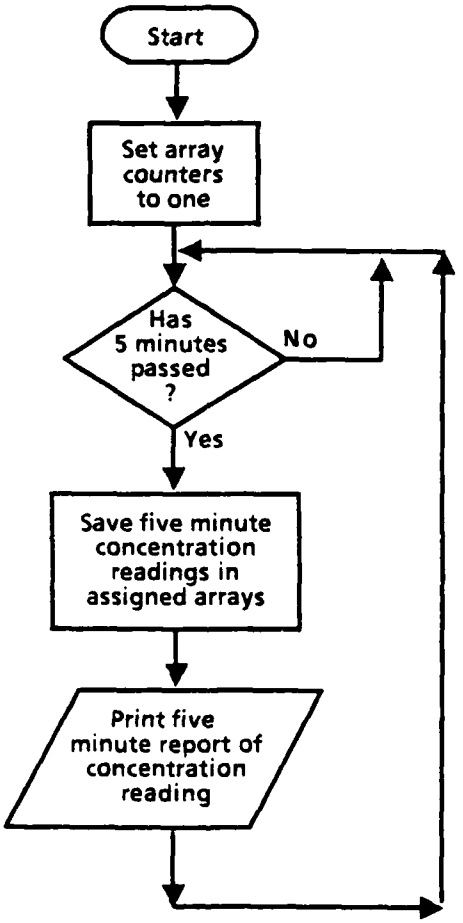


Figure B-7. Subtask - REPORT.

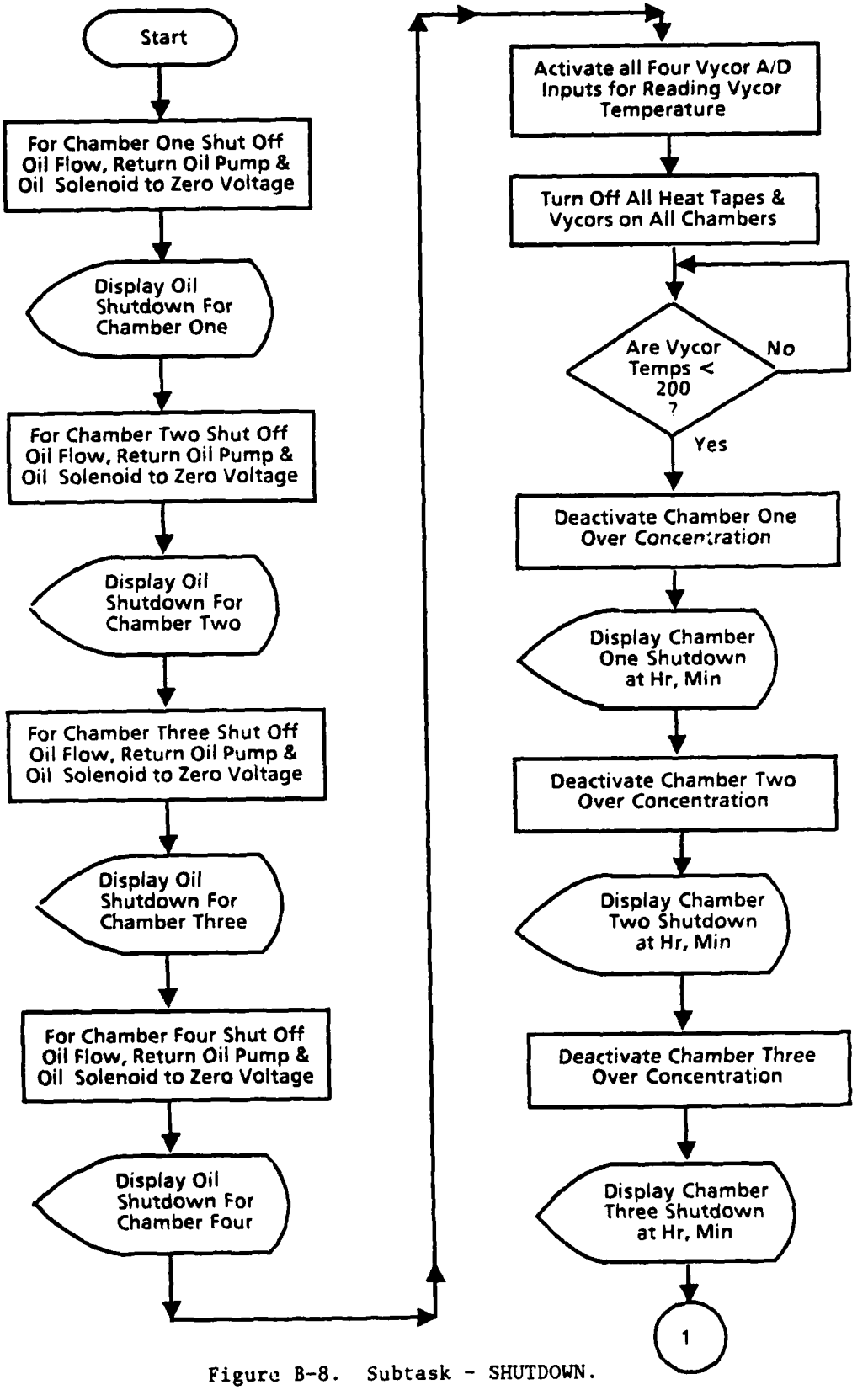


Figure B-8. Subtask - SHUTDOWN.

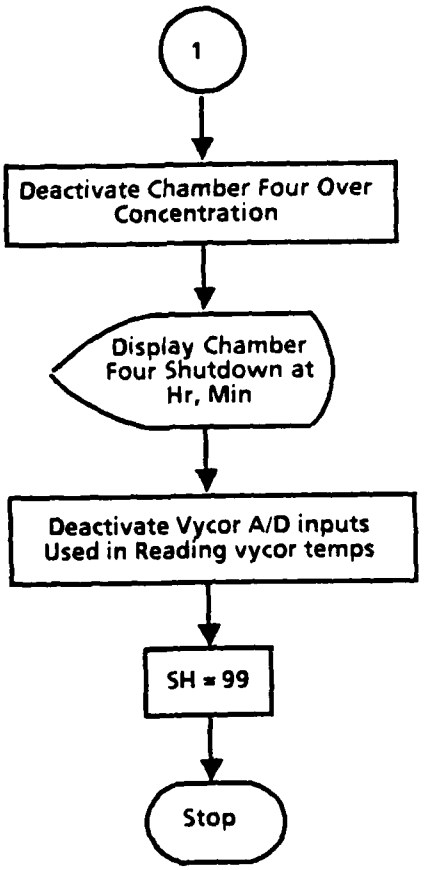


Figure B-8. Continued.

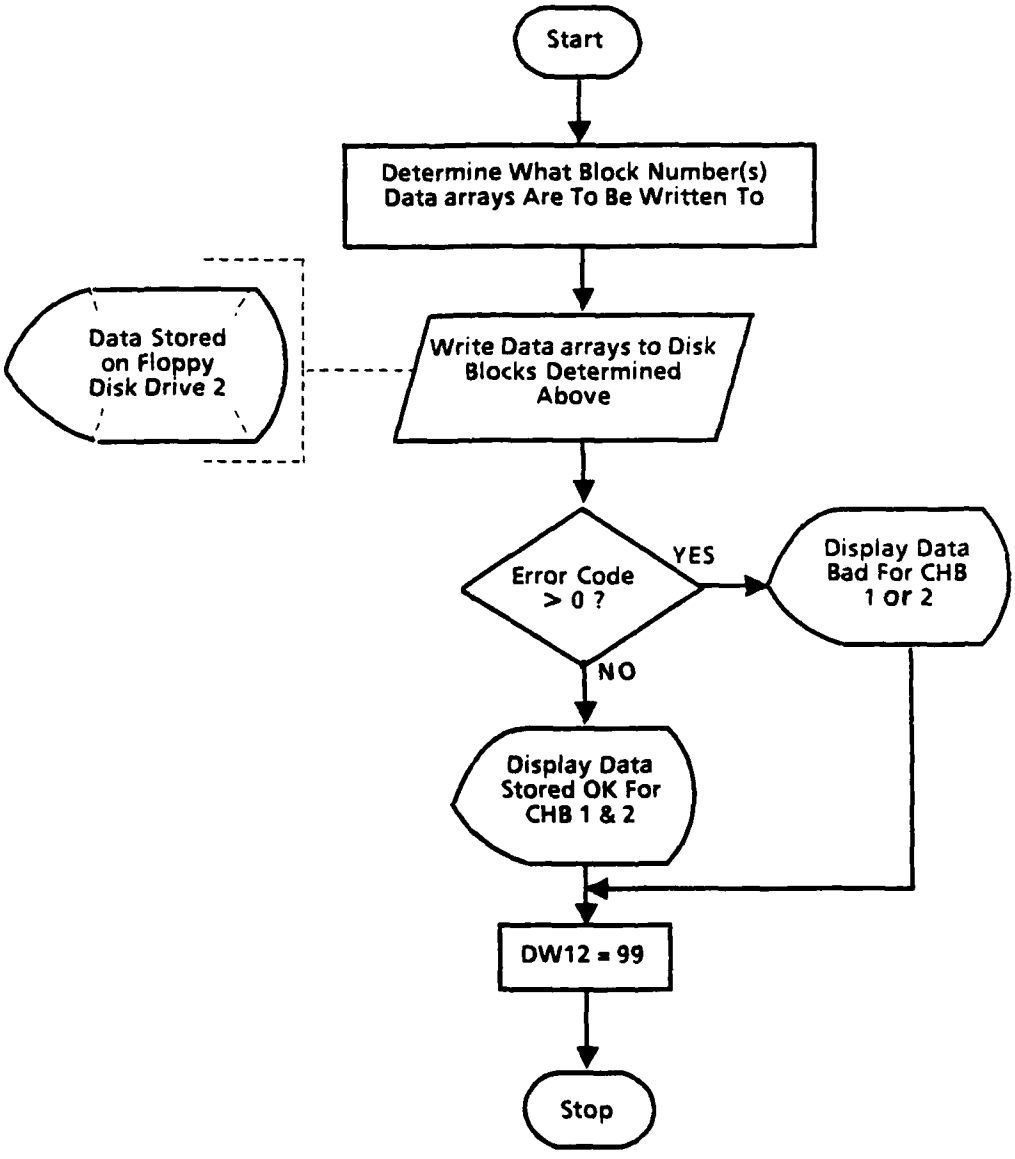


Figure B-9. Subtask - DWRT12.

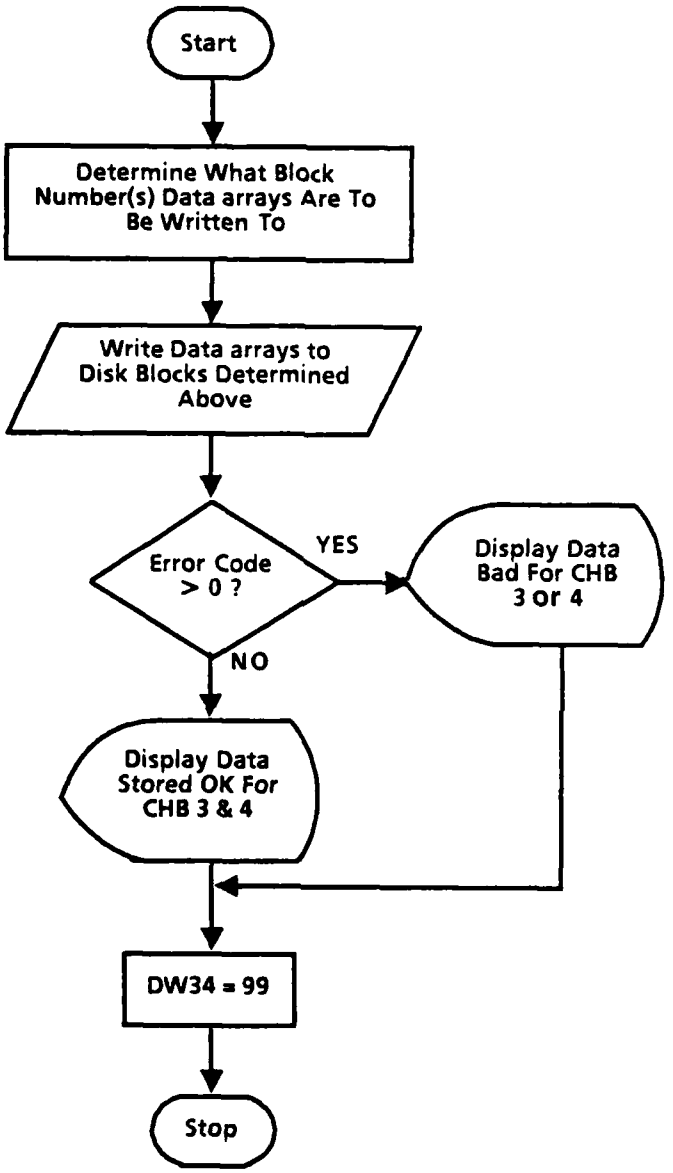


Figure B-10. Subtask - DWRT34.

APPENDIX C

SPECIFIC OPERATING PROCEDURES

REPLACEMENT OF THE INLET AIR FILTER

Charcoal adsorber elements should be changed or reactivated every 6 months. The high efficiency particulate air (HEPA) filters should be replaced only if the pressure drop across the filter is >0.5 in. H_2O.

Remove the filter housing from the generator shelf. The plastic connections on the filter housing are designed to allow easy removal of the assembly. The insulation jacket may be removed by carefully sliding it over the casing top. Replace the charcoal element with No. 80 mesh activated charcoal.

CHILLER OPERATION

A system is provided to condition room air to a level that is sufficient to counter the heat produced by the oil aerosol generators. This system consists of temperature monitors, a water chiller, two cooling coils, two control valves, a control panel with temperature limit alarms, and a circulating pump. The control panel is shown in Figure C-1.

Power is supplied to the chiller by a standard wall plug. When an adjustable thermostat in the water reservoir is energized, it controls operation of the compressor. This portion of the system operates independently from the water delivery system. The supply water temperature is adjusted by removing the chiller front panel and increasing or decreasing the thermostat setting.

The water delivery system is energized by the control power switch on the panel. The temperature monitor has sensors on the supply line leaving the chiller and on the return lines as they exit each cooling coil. The circulating pump has five speed settings and is adjustable from inside the chiller cabinet. Operation on speed 5 is recommended for most efficient temperature control. The pump is controlled by an off/automatic/on switch on the control panel. An indicator light turns "on" when the pump is energized. The "off" setting corresponds to no chilled water flow, and the "on" setting provides continuous chilled water circulation. In the automatic position, flow is on demand from either of the valve control modules. The valve control modules allow "off," "automatic," and "on" operation of three-way solenoid valves in the water return lines. Energizing the valve allows chilled water to flow through the associated cooling coil. De-energized flow bypasses the coil. The "on" setting provides continuous chilled water flow to the coil. The "automatic" position energizes the valve and pump on demand from the chamber thermostat. Temperature limit alarms may be set as desired to indicate chamber temperature extremes or equipment failure. The lower limit temperature alarm de-energizes the control valve to prevent further temperature drop. A single cooling coil is used for each pair of exposure chambers (1 and 2; 3 and 4).

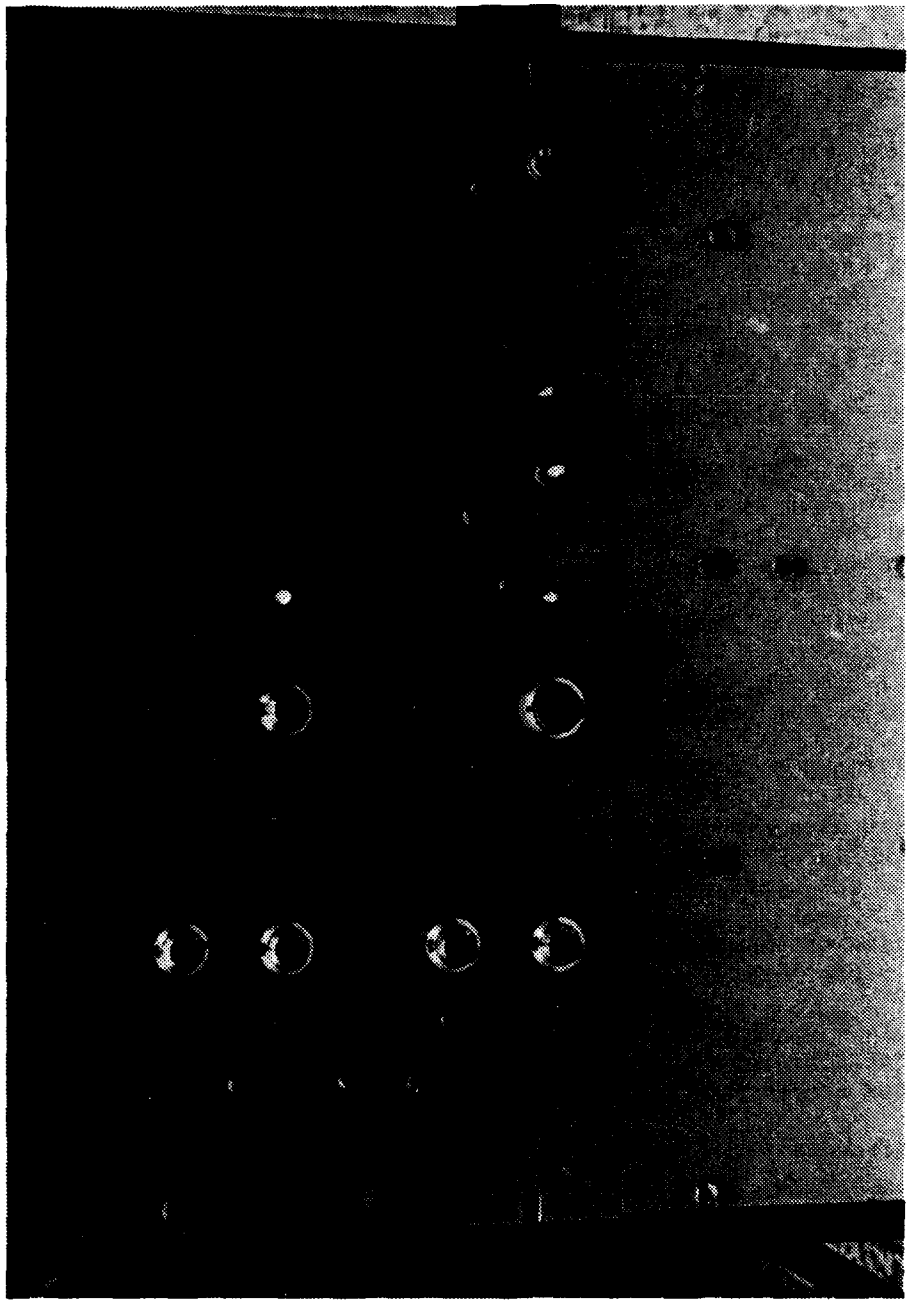


Figure C-1. Chiller control panel.

GLASS INLET DUCT CLEANING AND INSTALLATION

Oil collected on the inlet air duct system should be removed periodically to enhance system appearance and to avoid introducing liquid oil into the chamber. Because the collected oil is difficult to dissolve, remove the bulk oil with an absorbent cloth or laboratory napkin. Residual oil may be removed by washing the glassware in a soapy water solution.

There are two basic glassware configurations. The configuration of Chambers 1 and 4 is: PVC valve - tee - short nipple - generator tee - 90° elbow - short nipple - Y (45° arm) - short nipple - chamber flange. The configuration of Chambers 2 and 3 is: PVC valve - tee - generator tee - 90° elbow - Y (45° arm) - chamber flange. In both configurations, the dilution source is connected to the top of the Y. A thin oil film on the rubber portions of the glassware couplings enhances the assembly. The coupling should be secured firmly with a 7/16-in. nut driver.

BAFFLE CONE AND DIFFUSION PLATE INSTALLATION

The baffle cone and two diffusion plates are held in place by four threaded rod holders mounted to the chamber inlet flange. Wing nuts secure the cone and plates to the holders. The baffle cone top should be located approximately 2 in. below the air inlet. The small diffusion plate should be secured so that all four sides contact the slanting chamber walls. To prevent preferential flow around the large diffusion plate, a seal is necessary between the plate and the chamber wall; therefore, tape or a split rubber tubing flange is fitted onto the plate rim. The large diffusion plate should be secured so that all four sides make a positive seal against the chamber walls.

LIQUID NITROGEN SOURCE REPLACEMENT

Gaseous nitrogen is supplied to the aerosol generators from two liquid nitrogen cylinders located on the loading dock of the Inhalation Facility building. The nitrogen cylinders are equipped with pressure regulators preset at 55 and 45 psig. Contact facilities engineering for maintenance or modification of these preset pressures. The following procedure details cylinder replacement:

1. Close the gas valve on the cylinder, close the valve labeled "pressure building valve" on the cylinder, and close the on/off valve located downstream from the regulator.

2. Disconnect the supply line and replace the cylinder.

3. Reconnect the supply line to the gas port on the cylinder. Open the pressure building valve and wait for 30 min.

4. Open the gas valve on the cylinder, and open the off valve located downstream from the regulator.

NITROGEN FLOW MEASUREMENT

Nitrogen is used as a carrier gas in the oil aerosol generation system. The carrier gas performs three major functions. First, nitrogen provides an inert atmosphere for vaporizing the oil. Secondly, pressurized nitrogen prevents clogging of the oil injector by maintaining constant flow through it and thirdly, the oil vapor is carried by the nitrogen through the generator and injected into the chamber inlet air stream. To ensure proper operation of the generator, nitrogen flow through the generator, is one of the parameters that is routinely monitored from the exposure control panel.

Total nitrogen flow through the generator is measured by a wet test meter (GCA/Precision Scientific, 3 liter; GCA Precision Scientific, Chicago, IL). A calibration curve is constructed by plotting the actual flow versus the flow controller response. This calibration curve is used to maintain nitrogen flow at approximately 4.5 L/min.

CHAMBER AIR FLOW MEASUREMENT

Air flow through the exposure chambers is monitored continuously by measuring the pressure drop across a 0.875-in. orifice located at the chamber entrance. A calibration curve is constructed by plotting the actual flow versus the orifice pressure drop. This calibration curve is used to maintain the chamber flow at approximately 9.4 ft^3/min.

Actual flows are measured using a dry gas meter (Model 5M125TC; Dresser, Inc., Houston, TX). During flow measurement, the gas meter is placed in the chamber exhaust line between the balston filter and the primary exhaust blower.

CHAMBER AIR FLOW CONTROL SYSTEM

The four exposure chambers are specifically designed to allow independent control of the air flow through each chamber. The system consists of one fixed-speed inlet blower; four variable-speed and two fixed-speed exhaust blowers; a series of 2-in. gate valves; and flow monitoring equipment. The ultimate goals of -0.5 in. H_2O chamber pressure (versus ambient) and 7 to 11 ft^3/min chamber air flow are achieved by regulation of the inlet valves in conjunction with the variable speed exhaust blowers.

The inlet valves are adjusted to balance the pressure drops across the cooling coil and inlet filter. Once balanced, the variable-speed exhaust blower is adjusted to provide -0.5 in. H_2O pressure in the exposure chamber. Inlet and exhaust valves are provided for balancing pressure and flow through the two control chambers.

The following procedure is used to set up the chamber for -0.5 in. H_2O and 9.4 ft^3/min air flow. Refer to Figure C-2 for a schematic of the chamber air flow system.

1. Always adjust the chamber flow in order from Chamber 4 to Chamber 1.

2. Open valves (V) 1, 2, 3, 4, and 5.

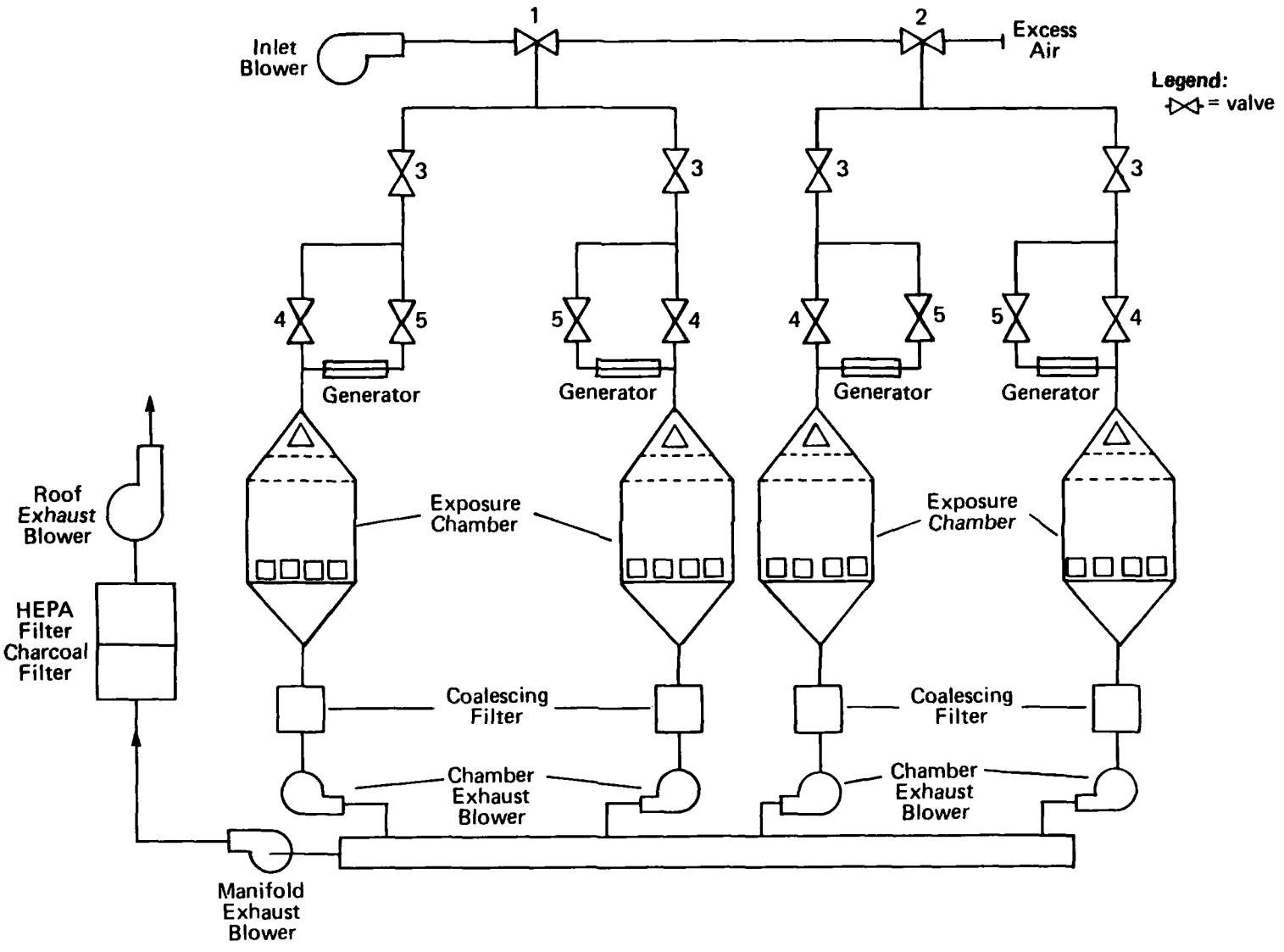


Figure C-2. Chamber airflow control schematic.

3. Energize the manifold exhaust blower, the chamber exhaust blower, and the inlet blower (the room exhaust blower is on continuously). Adjust the chamber exhaust blower until the chamber pressure gauge is at -0.5 in. H_2O.

4. Note the pressure drop across the orifice and determine the chamber flow from the performance curves.

5. To increase the flow, increase the chamber exhaust blower speed (this also increases the chamber pressure).

6. To decrease the chamber pressure, close V1 and/or V2, depending on which chamber(s) is/are being adjusted.

7. Balance out excess air by adjusting V3 on each pair of chambers (this may require slight adjustment of V1 and/or V2).

8. Repeat steps 3 through 6 until Chamber 4 is set. Proceed to Chambers 3, 2, and 1 in order. Include step 7 as needed.

9. Balance the dilution flow with the generator flow by closing V4 and/or V5 to achieve the desired relative velocity at the generator. Either V4 or V5 may be shut off but one valve must allow enough flow so that the total flow and pressure settings do not vary.

GENERATOR REMOVAL, DISASSEMBLY, AND INSTALLATION

The ORNL smoke generator (Figure 7) is the most critical subsystem of the aerosol system. Aerosol concentration and chemical composition stability depend upon proper generator operation. The generator requires periodic cleaning to remove fused petroleum deposits from the flow tube, thermocouples, and oil injector. The generator should be thoroughly inspected after each use to detect and replace heat stressed and worn components. The following procedures describe the removal, disassembly, and installation of the generator.

Generator Removal and Disassembly Procedure

1. The unit must be at ambient temperature for safe handling.

2. Disconnect all power connections: Vycor heater from junction box and heat tape from extension cord.

3. Remove the oil injector by loosening the 7/16-in. nut at the injector port until the injector needle slides freely in the holding assembly.

4. Slide the injector out of the holding assembly and gently (to avoid tube kinking) place it to the side. Allow the capillary tube to support the injector.

5. Repeat steps 3 and 4 for each of the three thermocouples inserted into the generator.

6. Remove the 9/16-in. nut on the nitrogen line. Remove the four 9/16-in. nuts from the pipe flange that connects the generator with the glass pipe.

7. Remove the generator.

8. Loosen the nut that holds the Teflon mounting sleeve on the Vycor heater, and slide the sleeve and heater from the generator tube. Carefully store the heater to prevent breakage.

9. To access the heat tape, you must remove the glass tape, wire, fiber insulation, wire, and insulation wrapping, in that order.

Generator Installation Procedure

1. This procedure assumes the availability of a complete generator assembly (i.e., intact heat tape, insulation, and installed Vycor heater). The glass inlet piping, including pipe flange, must be present.

2. Insert the generator gooseneck into the glass pipe with the outlet directed with the air flow. A Teflon gasket must be used between the generator flange and the glass pipe. The Vycor end of the generator rests on the bridge support provided.

3. Install four 9/16-in. nuts on the flange bolts. Be careful to tighten the bolts at the same rate to guard against warping the flange or breaking the glass pipe.

4. Remove the 9/16-in. nut, seal, and ferrule from the holding assembly. Slip the nut onto the thermocouple and wrap the fiber ferrule onto the thermocouple marked "heat tape." Insert the thermocouple into the holding assembly coupling nearest the glass pipe (No. 4). Approximately 3 in. into the generator, the tip of the thermocouple will touch the wall of the generator. Slide the thermocouple out of the tube approximately 1/2 in. Tighten the 7/16-in. nut to secure the thermocouple.

5. Repeat step 4 for the Vycor thermocouple. Insert the thermocouple into the No. 2 port approximately 1 in. until the tip touches the Vycor heater. Back the thermocouple out approximately 1/32 to 1/16 in. Secure the holding assembly.

6. Repeat step 5 for the limit thermocouple.

7. Repeat step 5 for the oil injector.

8. Connect the heat tape plug to the extension cord, connect the Vycor plug to the junction box, and connect the 9/16-in. nut to the nitrogen line.

9. Check the generator system by setting the nitrogen flow to 4.5 L/min, setting the chamber flow to 9.4 ft^3/min, and resetting the alarm under the limit monitor. Switch on the Vycor plug and heat tape. Set the Vycor plug to 600°C and the heat tape to 350°C.

10. When the temperatures stabilize, observe the Vycor plug and limit temperatures. One temperature is generally higher. The lower temperature indicates that the associated thermocouple needs to be adjusted slightly into the generator. Care should be taken to maintain a minimum clearance of 1/32 in. between the tip and the heater.

PARTICLE SIZE DETERMINATION

Particle-size data are collected with a cascade impactor from one location within the chamber. Probes are located 1 in. above each animal plane in the horizontal center position. The probes are connected to the impactor by two short lengths of 1/2-in. diameter tubing which pass through bulk head unions on the back of the chamber.

Instructions for assembly and operation of the impactor, including data presentation and analysis, are given in the "Operating Manual for Andersen Samplers, Inc.," (Andersen Samplers, Inc., Atlanta, GA).

Results of the data analysis are plotted on a 2 log cycle probability graph for the determination of the mass median aerodynamic diameter and associated standard deviation (Sigma g).

SPACIAL DISTRIBUTION ANALYSIS

Distribution testing was performed on two horizontal planes. Probes 1 through 5 were located on the top plane (1 in. above the upper animal shelf). Probes 6 through 10 were located on the bottom plane (1 in. above the lower animal shelf). See Figure C-3.

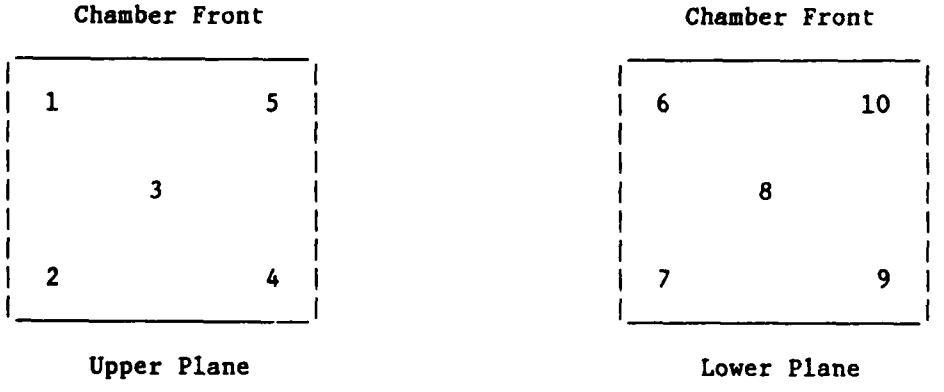


Figure C-3. Exposure chamber planes.

Absolute concentrations were determined at each point by filter sample analysis. Filter samples were collected using a filter-limiting orifice/vacuum arrangement. Limiting orifice flow rates were determined before each test run. The constant flow range of the limiting orifices was -18 to -26 in. Hg vacuum. Vacuum was supplied by a piston vacuum pump operated at -28 in. Hg. A five-port vacuum manifold allowed the simultaneous collection of five filter samples. Aerosol mass concentration was determined gravimetrically from the filter samples.

Distribution tests were performed as discussed in Appendix D (at 0.5, 1.0, 2.0, and 4.0 mg/L). Using 4.9 L/min limiting orifices, the required sample time for selected concentrations is given in Table C-1.

The tests were performed in sequence from 0.5 mg/L to 4 mg/L. Once the concentration level was stable at the desired test concentration, three sets of filter samples were taken consecutively from the top plane. The filter holders were moved and three sets of filter samples were taken from the bottom plane. This process was repeated for each of the test concentrations.

TABLE C-1. DISTRIBUTION TEST PARAMETERS

Concentration (mg/L)	Sample Time (min)
0.5	8
1.0	5
2.0	3
4.5	2

AEROSOL CONCENTRATION DETERMINATION

Aerosol concentrations are determined by filter sample analysis. Aerosol samples are collected on 47-mm glass fiber filters using a filter-limiting orifice/vacuum arrangement. Mass collections are quantitated gravimetrically using a Mettler analytical balance having a stated accuracy of ±0.1 mg. Probe points are connected to the filter collection system by 1/2-in. diameter tubing. Vacuum is supplied by a piston vacuum pump operated at -28 in. Hg vacuum. Orifice flow rates are determined weekly.

During normal exposure operation, aerosol concentrations are determined 1 in. above each horizontal animal plane in the center of the planes. Sample times should be precalculated for the desired concentration to provide a total mass collection in the range of 10 to 30 mg of oil per filter collection.

Continuous aerosol concentrations are recorded by the RAM-1 aerosol sensors. Prior to use, a calibration curve is constructed by comparing sensor output with filter concentration determinations. The calibration curve is

prepared for the range of approximately ±20 percent of desired concentration. Filter samples taken at hourly intervals during the exposure provide a quality control for the RAM-1 sensor calibration.

BULK OIL STORAGE AND HANDLING

Five-gallon sealed containers of unused oil are stored in the refrigerator located in the receiving area. The refrigerator temperature is maintained at 5°C. Before the oil is used, it is transported to the exposure facility and mechanically mixed by rotation for 12 hours.

After passing through the aerosol generator, excess oil collected from the Balston filter and generator drip reservoir is removed, packaged in plastic containers, and discarded in the waste receptacle.

APPENDIX D
PRELIMINARY DISTRIBUTION STUDY - FOG OIL CHAMBER CHARACTERIZATION

STUDY I: HOMOGENEITY OF AEROSOL CONCENTRATION IN CHAMBER 4 WITH ANIMALS ABSENT

Fog oil was generated and sampled at 10 points within the chamber. These were the four corners and a center point on each of the two shelves. The study was repeated six times using different concentrations. No animals were in the chamber.

The data were analyzed using a three way analysis of covariance (ANCOVA--The procedure GLM of the SAS software referenced in the User's Guide. (SAS Institute, Inc. 1982. SAS User's Guide: Statistics, 1982 Edition. SAS Institute, Inc., Cary, NC.) was used to perform the statistical analysis.) The sample taken at the center point was used as the covariate for each shelf at each concentration. The parameter estimate for this variable was 1.002 with a standard error of 0.125. A t test for the null hypothesis that this value equals 1 was not rejected. This implies that the center point adequately represents the concentration for a given shelf and that this relationship is reproducible over the range of doses tested (2.87 mg/L to 7.68 mg/L).

In the ANCOVA, the residual variability not explained by the covariate is examined for consistent factor effects. Three factors were analyzed to test for consistent patterns of deviation within the chamber. These were level (shelf 1 or shelf 2), position (front or back), and side (right or left). All possible interactions were also tested. A significant three way interaction was detected, although no other effects were found.

Using t tests, the effects could be detected as a significant difference between the front and back corners on the right side of shelf 2 with a P value of 0.057 as well as four other differences at the 0.08 to 0.10 level. However, using appropriate multiple comparison tests, such as Duncan's multiple range procedure, and setting the P level at 0.05, no differences could be detected. Furthermore, the maximal difference of 5.4 percent that was detected as being

statistically significant is well within the exposure specifications achievable with state-of-the-art inhalation technology.

The means (mg/L), adjusted for concentration, are presented schematically in Figure D-1. The standard error for the adjusted means was 0.09. The sample size was 6.

Shelf 1			Shelf 2		
	(B)			(B)	
	4.83	5.04		4.99	4.80
(L)		(R)	(L)		(R)
	5.03	5.03		4.88	5.06
	(F)			(F)	

Figure D-1. Adjusted means for Study I.

STUDY II: HOMOGENEITY OF AEROSOL CONCENTRATION IN CHAMBER 4 WITH ANIMALS PRESENT

In Study II, a single concentration of fog oil in approximately the middle of the range of doses tested in Study I, was generated and sampled as in Study I. The procedure was replicated four times. A full complement of animals was loaded into the chamber.

Because a single concentration of fog oil was generated, the statistical technique used in Study I, ANCOVA, was not appropriate for the analysis in Study II. Instead, the data were analyzed with a three way analysis of variance (ANOVA) with blocking factor. Using the center point as a control, the data were transformed and expressed as difference from control.

The actual means and standard errors are presented in Figure D-2. The sample size for all points was four. Note that, unlike Figure D-1, the means (mg/L) in Figure D-2 are not adjusted for the center point.

Shelf 1			Shelf 2		
	(B)			(B)	
	4.28	3.77		4.08	3.69
	(0.10)	(0.31)		(0.06)	(0.24)
(L)		(R)	(L)		(R)
	4.31	4.48		4.06	4.27
	(0.12)	(0.07)		(0.10)	(0.04)
	(F)			(F)	

Figure D-2. Actual means and standard errors.

No effects due to replication were found. A position (decreased values in the back) main effect ($P < 0.05$) and a position by side interaction ($P < 0.05$) were significant. The interaction effect was subtested using Duncan's multiple range procedure. Results indicated that the concentration in the back right corner was significantly lower than the concentration in any of the other three corners.

The maximal difference in concentration was just under 20 percent. While this may be acceptable in some cases and can be randomized in long term exposures, the engineers examined whether the variability can be reduced further. Additional tests for leaks were conducted but none were found. Inspection of the baffle at the top of the chamber revealed that not all of the aerosol was being forced through the baffle. This situation was corrected and additional studies were conducted.

STUDY III AND IV: HOMOGENEITY OF A CONCENTRATION IN CHAMBER 4 WITH AND WITHOUT ANIMALS

As in the initial two studies, fog oil was generated and sampled at ten points within the chamber. These were the four corners and a center point on each of the two shelves. In Study III, samples were taken with the chamber loaded with cages, but without animals; while in Study IV, animals were loaded into the chamber.

In both studies, four concentrations of fog oil were generated, ranging from 0.5 to 4.5 mg/L. This range of concentrations was selected based on the preliminary results from the LC_{50} studies (3).

The data were analyzed using a three way analysis of covariance (ANCOVA). Using the center sample as the covariate, the parameter estimate was tested for quality to one. The residual variability was then examined for consistent patterns of deviation within the chamber that could be attributed to the three factors. Results from the two studies are discussed below.

STUDY III: ANIMALS ABSENT

The parameter estimate for the covariate was 1.02 ± 0.005. The t statistic testing for equality to one was rejected ($P < 0.01$). In spite of the statistical significance, the center point still adequately represents the concentration and the distortion is not too great over the relevant range. It represents a maximal difference of 2.0 percent which is well within engineering technology specifications. Among the factors, both side ($P < 0.01$) and position ($P < 0.05$) main effects were detected. The maximal difference, occurring from front to back, was 2.4 percent, which again is well within technological constraints.

The means (mg/L), adjusted for concentration, are presented schematically in Figure D-3. The standard error was 0.002. The sample size was 4.

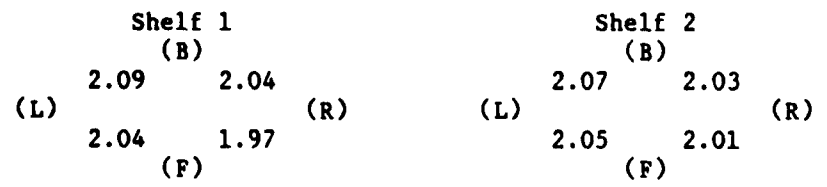


Figure D-3. Means (mg/L) adjusted for concentration (animals absent).

STUDY IV: ANIMALS PRESENT

The covariate parameter estimate in this study was 1.02 ± 0.01. Based on a t statistic, the test for equality to one was not rejected. This finding, coupled with the results from Study I, suggest that the statistical significance detected in Study III reflects system noise, and that the center point may be used to represent the chamber concentration over the relevant range.

Analysis of factor effects indicated a side by position interaction. When this was subtested with the appropriate corrections for multiple comparisons, however, no individual differences among the means could be detected.

The means (mg/L), adjusted for concentration, are presented schematically in Figure D-4. The standard error was 0.05.

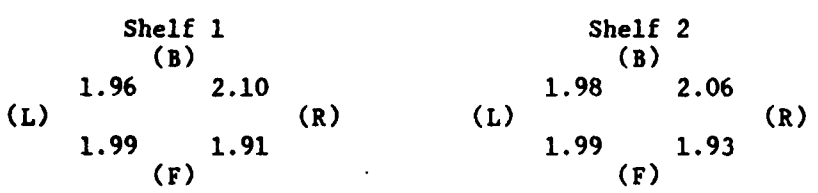


Figure D-4. Means (mg/L), adjusted for concentration.

In summary, when aerosol was generated in the chamber without animals, the estimate of the covariate was found to be statistically different from one. Additionally, there were main effect differences from front to back and from right to left. The maximal difference from any one of these effects was 2.4 percent which is well within engineering feasibility constraints. Furthermore, when the study was repeated, using similar concentrations, in the chamber loaded with animals, none of these differences were found. The test for the covariate's equality to one could be rejected. Among the factors, one interaction effect appeared to have statistical significance. But differences among the means could not be detected when subtesting was performed.

STUDY V: HOMOGENEITY OF AEROSOL CONCENTRATION IN CHAMBERS 1 THROUGH 3 WITH ANIMALS ABSENT

Studies I to IV were detailed analyses of the distribution of fog oil in one chamber, Chamber 4. In this study, the remaining three chambers were tested to determine whether the fog oil was uniformly dispersed within each chamber.

As in the other studies, fog oil was generated and sampled at 10 points within the chamber. These were the four corners and a center point on each of the two shelves. No animals were in the chamber.

Two concentrations of aerosol were generated in each chamber: the low was approximately 0.7 mg/L and the high concentration about 7.0 mg/L. Although the

concentrations were similar, efforts were not made to keep them identical in all chambers.

The data for each chamber were analyzed separately using three way analyses of covariance (ANCOVA). Using the center sample as the covariate, the parameter estimate was tested for equality to one. The residual variability was then examined for consistent patterns of deviation within the chamber that could be attributed to the three factors. Results of the analyses are discussed below.

No effects due to the factors or their interactions were found in any of the chambers except in Chamber 2 where the concentration of fog oil was higher ($P = 0.08$) on the right side than on the left. This presented a four percent increase, however, which is within achievable exposure specifications. In all three chambers, the hypothesis that the coefficient of the covariate was equal to one was not rejected. The adjusted means and standard error for each chamber are presented in Figure D-5.

CHAMBER 1
(std. err. = 0.09)

SHELF 1			SHELF 2		
	(B)			(B)	
3.99		3.93	3.99		3.88
(L)		(R)	(L)		(R)
4.01		3.89	3.88		3.74
	(F)			(F)	

CHAMBER 2
(std. err. = 0.12)

SHELF 1			SHELF 2		
	(B)			(B)	
4.20		4.27	4.11		4.15
(L)		(R)	(L)		(R)
4.05		4.41	4.02		4.26
	(F)			(F)	

CHAMBER 3
(std. err. = 0.08)

SHELF 1			SHELF 2		
	(B)			(B)	
4.23		4.20	4.34		4.26
(L)		(R)	(L)		(R)
4.18		4.07	4.24		4.18
	(F)			(F)	

Figure D-5. Means (mg/L), adjusted for concentration - multiple chambers.

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